MOLECULAR DOCKING AND SYNTHESIS OF 1, 2, 4 - TRIAZIN ANALOGUE OF DICLOFENAC AS POTENTIAL LIGAND FOR CHLORPROMAZINE INDUCED PARKINSON'S IN RAT MODEL

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

Chennai-600032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
PHARMACOLOGY

Submitted by

S. POORANA PUSHKALAI

REG. NO.: 261525552

Under the Guidance of

Mr. P. SUDHAKAR, M. Pharm.,

Assistant Professor, Department of Pharmacology

DEPARTMENT OF PHARMACOLOGY

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY, ELAYAMPALAYAM, TIRUCHENGODE-637205, NAMAKKAL DISTRICT, TAMIL NADU.

OCTOBER-2017

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Elayampalayam, Tiruchengode- 637205

Namakkal (DT.), Tamilnadu.

Phone: 04288-234417

Fax: 04288-234417

Dr. G. MURUGANANTHAN, M. Pharm., Ph.D.,

PRINCIPAL

CERTIFICATE

This is to certify that the Dissertation entitled "**Molecular Docking and Synthesis of 1, 2, 4 – Triazin Analogue of Diclofenac as Potential Ligand for Chlorpromazine Induced Parkinson's in Rat Model**" submitted to **The Tamil Nadu Dr. M.G.R. Medical University,** Chennai, is a bonafide project work of **S. POORANA PUSHKALAI (Reg. No: 261525552)** carried out in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode for the partial fulfillment for the degree of Master of Pharmacy under the guidance and direct supervision of **Mr. P. SUDHAKAR, M. Pharm.,** in the Department of Pharmacology during the academic year of 2016-2017.

Date:

Place: **Dr. G. MURUGANANTHAN, M. Pharm., Ph.D.,**

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Elayampalayam, Tiruchengode- 637205

Namakkal (DT.), Tamilnadu.

Phone: 04288-2344

Fax: 04288-234417

Dr. V. VINOTH PRABHU, M. Pharm., Ph.D.,

Head, Department of Pharmacology

CERTIFICATE

This is to certify that the Dissertation entitled "**Molecular Docking and Synthesis of 1, 2, 4 – Triazin Analogue of Diclofenac as Potential Ligand for Chlorpromazine Induced Parkinson's in Rat Model**" submitted to **The Tamil Nadu Dr. M.G.R. Medical University,** Chennai, is a bonafide project work of **S. POORANA PUSHKALAI (Reg. No: 261525552)** carried out in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode for the partial fulfillment for the degree of Master of Pharmacy under the direct guidance and supervision of **Mr. P. SUDHAKAR, M. Pharm.,** in the Department of Pharmacology during the academic year of 2016-2017.

Date:

Place: **Dr. V. Vinoth Prabhu, M. Pharm, Ph. D.,**

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Elayampalayam, Tiruchengode- 637205

Namakkal (DT.), Tamilnadu.

Phone: 04288-234417

Fax: 04288-234417

Mr. P.SUDHAKAR, M. Pharm.,

Assistant Professor, Department of Pharmacology

CERTIFICATE

This is to certify that the Dissertation entitled "**Molecular Docking and Synthesis of 1, 2, 4 – Triazin Analogue of Diclofenac as Potential Ligand for Chlorpromazine Induced Parkinson's in Rat Model**" submitted to **The Tamil Nadu Dr. M.G.R. Medical University,** Chennai, is a bonafide project work of **S. POORANA PUSHKALAI (Reg. No.: 261525552)** carried out in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode, for the partial fulfillment for the degree of Master of Pharmacy under my direct guidance and supervision during the academic year of 2016-2017.

This work is original and has not been submitted earlier for the award of any other Degree or Diploma of this or any other university

Date:

Place: **Mr. P.SUDHAKAR, M. Pharm.,**

DECLARATION

This is to certify that the Dissertation entitled "**Molecular Docking and Synthesis of 1, 2, 4 – Triazin Analogue of Diclofenac as Potential Ligand for Chlorpromazine Induced Parkinson's in Rat Model**" submitted to **The Tamil Nadu Dr. M.G.R. Medical University,** Chennai, is a bonafide project work carried out myself **S. POORANA PUSHKALAI (Reg. No: 261525552)** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode for the partial fulfillment for the degree of Master of Pharmacy under the guidance and direct supervision of **Mr. P. SUDHAKAR, M. Pharm.,** in the Department of Pharmacology during the academic year of 2016-2017.

Hereby I declare that this work embedded in the thesis is original and not submitted in part or full for any other degree of this or any other university.

S. POORANA PUSHKALAI

(REG. NO: 261525552)

EVALUATION CERTIFICATE

This is to certify that the Dissertation entitled "**Molecular Docking and Synthesis of 1, 2, 4 – Triazin Analogue of Diclofenac as Potential Ligand for Chlorpromazine Induced Parkinson's in Rat Model**" submitted to **The Tamil Nadu Dr. M.G.R. Medical University,** Chennai, in partial fulfillment for the degree of Master of Pharmacy. This was carried out by **S. POORANA PUSHKALAI (Reg. No: 261525552)** under the guidance and direct supervision of **Mr. P. SUDHAKAR, M. Pharm.,** in the Department of Pharmacology, Swamy Vivekananda College of Pharmacy, Tiruchengode for the during the academic year of 2016-2017.

Internal Examiner **External Examiner**

Examination centre: Swamy Vivekanandha College of Pharmacy,
Elayampalayam, Tiruchengode.

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

First and foremost, I bow down before **LORD ALMIGHTY**, the most humane, the most indulgent. All praise and thanks are due to him who had bestowed us with health and courage during the course of my work and throughout my life till this every second.

I feel it a great honour to express my deep sense of gratitude and indebtedness to my wonderful project guide **Mr. P. SUDHAKAR.P, M. Pharm.,** Assistant Professor**,** Department of Pharmacology, for his invigorate guidance, felicitous advice, constructive help, suggestions, encouragement and friendly support during the whole course of my work, punctilious and valuable hints with energizing criticism during the course of dissertation work. Indeed without her guidance and optimistic approach this project wouldn't have been a successful one.

It is difficult to overstate my gratitude to **Dr. G. MURUGANANTHAN, M. Pharm., Ph.D.,** and Principal of this institution. His enthusiasm and integral view on research and his mission for providing 'only high-quality work and not less', has made a deep impression on me. I owe him lots of gratitude for having me shown this way of research.

I feel it a great to express my deep sense of gratitude and indebtedness to my Head of Department of Pharmacology of this institution **Dr. V. VINOTH PRABHU, M.Pharm., Ph.D.,** thanking for his support encouragement and his constructive ideas at each and every stage of the project which were the driving forces for me to complete this thesis.

I submit my sincere and respectful regards to our beloved chairman and secretary Vidyaratna, Rashtriya rattan, Hind rattan**, Prof. Dr. M. KARUNANITHI, B.Pharm, M.S., Ph.D. D. Litt,** who provided all the facilities in this institution enabling us to do a work of this magnitude.

I also extend my sincere equal thanks to **Mr. R. ANAND, M.Pharm, Ms. S. PRIYADHARSHINI, M.Pharm,** and **Ms. K.B. SUCHITHRA, B.Pharm,** Department of Pharmacology for their valuable advice and friendly support throughout the course of study.

I remain my sincere thanks to non-teaching staff **Mr. V. KARUNAKARAN, Mrs. L. SATHIYA,** and **Ms. S. GOWRI**, Department of pharmacology, SVCP Animal house care taker **Mr. S. MARI** and Library staff **Mrs. K. SHARMILA, Mr. P. SAHAYARAJU** for help to finish the project work in a successful manner.

I am immensely grateful to staff of all other departments, and all nonteaching staffs, Swamy Vivekanandha college of Pharmacy, Tiruchengode, for their garnered blessings showered on me throughout my academic career.

I owe my heartfelt gratitude to my respected **Family members**, I take this privilege and pleasure to acknowledge my hero **Mr. K. SARAVANAN, B.E,** my heroine **Mrs. S. PREMA**, my princess **Ms. S. VAISHNAVI,** my king **Mr. N. KRISHNAN** and my Queen **Mrs. K. BABY Late** whose unconditional love, support and encouragement shaped up my life. Without their moral support, I am nothing and I dedicate all my achievements at their feet.

FRIENDS are treasures to me and it is very difficult to overstate my thanks to M. **SUGANESWARI, KM. PREETU SHUKLA, S. HARIPRIYA** and all my friends. It has been my happiest time to study, discuss, laugh and play with them all. I express my whole hearted thanks to my friends.

I would like to thanks **THE TAMIL NADU DR. M. G. R. MEDICAL UNIVERSITY** for providing a nice environment for learning.

My sincere gratitude and appreciation goes to all who have directly or indirectly contributed to my project successfully.

S. POORANA PUSHKALAI

(REG. NO: 261525552)

ABSTRACT

ABSTRACT

Title	:	**Molecular Docking and Synthesis of 1, 2, 4–Triazin Analogue of Diclofenac as Potential Ligand for Chlorpromazine Induced Parkinson's in Rat Model.**
Name	:	**S. POORANA PUSHKALAI**
Register Number	:	**261525552**
Degree to which submitted	:	**Master of Pharmacy in Pharmacology**
Guide	:	**Mr. P. Sudhakar, M. Pharm.,**
Department	:	Department of pharmacology
College	:	Swamy Vivekanandha College of Pharmacy, Tiruchengode.
University	:	**Tamil Nadu Dr. M.G.R Medical University, Chennai- 32.**
Year	:	2016-2017

Aim:

Parkinson's disease (PD) is a common neurodegenerative disorder, which is characterized by slowly progressive loss of dopaminergic neurons associated with substantial. The present study was aimed for molecular docking and synthesis of 1, 2, 4–Triazin analogue of diclofenac as potential ligand for chlorpromazine induced Parkinson's in rat model.

Materials and methods:

Twenty four Albino Wistar male Rats weighing 250-270 gm were randomly assigned to four groups, each group contain 6 animals. **Group I** Vehicle group- Received 1% Gum acacia + distilled water p.o for 14 days, **Group II** Negative control group- Received Chlorpromazine 3mg/kg, i.p. (dissolved with 1% gum acacia in distilled water suspension), **Group III** Standard group - Received Chlorpromazine (3mg/kg/day), i.p. + Diclofenac (10mg/kg/day) p. o., **Group IV** treatment group- Received Chlorpromazine (3mg/kg/day) i.p. + 1,2,4-Triazin derivative of Diclofenac at dose of 30mg/kg, p. o. Parkinson's disease (PD) was induced by intra peritoneal injection of chlorpromazine 3mg/kg, i. p (dissolved with 1% gum acacia in distilled water suspension) daily for a period of 14 days. The 1, 2, 4-Trianine analogue of diclofenac was synthesised and evaluated against chlorpromazine induced PD by monitoring *in vivo behavioural* paramerters like muscle coordination, cognitive performance, catalepsy activity, Biochemical estimation of SGPT, SGOT, ALP, Total bilirubin, urea, creatinie and brain antioxidant levels, Acetylcholine, dopamine. Changes were confirmed by Histopathological studies.

Results:

Molecular docking of 1, 2, 4- Triazin derivative of diclofenac, binding scores of designed ligand was 1-10 scores with D_3 protein, DDC, $AA_{2A}R$, MAPK and MAO-B enzymes ranging from -6.35 to -5.64 Kcal/mol, -6.86 to -5.81 Kcal/mol, -6.11 to -5.02, -8.67 to -4.63 Kcal/mol, and -10.25 to -6.76 Kcal/mol respectively. Treatment group shows significant increase the body weight, feed intake, Locomotion action, muscle coordination, cognitive performance and dopamine level, also decrease in muscle rigidity, oxidative stress and cholinergic over activity.

Conclusion:

1, 2, 4 -Triazin derivative of diclofenac shows significantly anti Parkinson's activity against chlorpromazine induced PD rats with milder GI toxicity as compared to the diclofenac treatment. Further clinical data are required to explore this Analogue of diclofenac as Potential Ligand for improving the status of PD patients.

ABBREVIATIONS

ADP	Alkaline phosphate
AD	Alzheimer's disease
COMT	Catechol-o-methyl-transferase
CPCSEA	Committee for the purpose of control and supervision on experiments on animal
CNS	Central nervous system
DA	Dopamine
DNA	Deoxy ribo nucleic acid
FTLD	Frontotemporal dementia
GDNF	Glial-derived neurotrophic factor
GPI	Globus pallidal segment
GABA	Gamma aminobutyric acid
L-AAD	L-Amino acid decarboxylase
MSA	Multiple system atrophy
MRC	Medical research council
MPTP	4-methyl-4-Phenyltetrahydro pydrine
NMDA	N-methyl-D-aspartate
NSAIDs	Non steroidal anti-inflammatory drugs
NO	Nitric oxide
NDD	Neurodegenerative disorders
PDB	Protein data bank
PG	Prostaglandin
PD	Parkinson's disease
RCSB	Research collaborator for structural bioinformatics
SNPC	Substantia nigra pars compacta
SGPT	Serum glutamate pyruvate
TX	Thromboxane
TCA	Tri chloro acetic acid
WHO	World health organization

CONTENT

Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

CHAPTER-1

INTRODUCTION

CHAPTER - 1

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative brain disorder. It is characterized by diminished facial expression, stooped posture, slowness of voluntary movement, festinating gait (progressively short- ended, accelerated steps), rigidity, and a "pill- rolling|" tremor but also of many other central and peripheral neuronal systems[1]. More than 1 million people in the United States are affected by the disease. It usually begins after 50 years of age; most cases are diagnosed in the sixth and seventh decade of life. PD, the most common from Parkinsonism, is named after James Parkinson, a British physician who first described the disease in 1817 on the "shaking palsy" [2].

The aetiology of PD is unknown but several mechanisms have been proposed including environmental toxins, oxidative stress and neuro inflammation. The involvement of such dopamine and non-dopaminergic systems is responsible for the occurrence of the motor and non-motor Parkinson's symptoms. Non-motor symptoms and their management are now recognized as an important unmet need in PD. They affect the great majority of PD patients and may sometimes be more closely related to reduce quality of life than the core motor symptoms [3].

A pathological classification of Lewy body diseases includes the loss of pigmented dopaminergic neurons and the presence of Lewy bodies. Progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which project to the striatum (the nigrostriatal pathway), results in the loss of dopaminergic functions in individuals with PD. PD is characterized by the accumulation of intracellular protein aggregates, Lewy bodies and Lewy neuritis which composed of primarily protein α-synuclein. These neurons contain the neurotransmitter DA, and their projecting nerve fibers reside in the striatum [4, 5].

In Dopamine loss in the basal ganglia triggers prominent secondary morphological changes and its depletion also triggers changes in the density and sensitivity of dopamine receptors [6]. DA are two types of D1 (excitatory type) and D2 (inhibitor type), influence motor activity in the extra pyramidal system. Components of this system include the basal ganglia, which involves the internal globus pallidal

segment (GPi) of the ventral striatum, and the pars reticulata portion of the substantia nigra (SNpr). The loss of dopamine in the striatum of PD patients results in increased activity in the GPi/SNpr circuits and subsequent gamma aminobutyric acid (GABA) dysfunction, leading to inhibition of the thalamus [7, 8].

Non steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs in modern medicine. NSAID use is however associated with several serious side effects, with considerable associated morbidity and mortality [9]. The best-known mechanism of action of NSAIDs is associated with inhibition of prostaglandin (PG) and thromboxane (TX) production by interaction with cyclooxygenase (COX-1 and COX-2) enzymes. It is now known that there are two structurally distinct forms of the cyclo-oxygenase enzyme (COX-1 and COX-2). COX-1 is a constitutive member of normal cells and COX-2 is induced in inflammatory cells. Inhibition of COX-2 activity represents the most likely mechanism of action for NSAID-mediated analgesia, while the ratio of inhibition of COX-1 to COX-2 by NSAIDs should determine the likelihood of adverse effects [10].

NSAIDs and neurological diseases is an important mechanism in defence responses to progression of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and other neurological disorders. The over-expression of COX-1 and COX-2 has been found in the central nervous system of patients with ischemic, traumatic brain injury and those with neurodegenerative diseases such as AD and PD, which indicates that COX mediated neuroinflammation, is a critical component in neuronal degeneration. As reactive microglia were first observed in the vicinity of degenerating dopaminergic substantia nigral regions of post-mortem brains taken from patients with Parkinson's disease. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce dopaminergic neuron degeneration in animal models of PD [11].

Non steroidal anti-inflammatory drugs (NSAIDs) readily cross blood brain barriers and inhibits microgial released cyclo-oxygenases and other cytokines. Investigated that diclofenac along with sertraline signification inhibit inflammatory cytokines like TNF-α, interleukins and promote the release of IL -10 which is an anti-inflammatory in nature. In addition, different animal experimental studies have shown that COX-2 inhibitors are reversed in rodents with decrease in inflammatory

cytokinase. Diclofenac is more potent in inhibiting COX-2 than COX-1 isoenzymes [12]. A new anti-Parkinson's drug diclofenac is effective in animal models and in patients with early stage of PD. It has a complex mechanism of action that involves uncompetitive, low affinity NMDA receptor open-channel blocking. Additionally, diclofenac exhibits anti radical and immunuotropic effects.

1, 2, 4-Triazine derivative as antagonists of Adenosine A_2 receptor is expressed in the basal ganglia where it functionally opposes the actions of the dopamine D2 receptor. i.e., inhibition of the A_2 receptor leads to enhancement of D2 receptor function [13].

Several previous studies have suggested that diclofenac may elicit appreciable GI irritation, bleeding and ulceration produced. Synthetic approaches based upon chemical modification of diclofenac have been taken with the aim of improving safety profile and in turn therapeutic window. Carboxylic group is a major reason for the GI toxicity of diclofenac. Structural replacement of carboxyl group may reduce the GI toxicity. Previous study reported that 1, 2, 4-Triazin derivative may possess appropriate action on Parkinson's action. Based on above, fact this study was aimed for replacement of carboxylic group with 2-Chloroacetamide to produce the 1, 2, 4 –Triazin derivative of diclofenac as potential target for PD [14].

Nowadays, the discovery of new drugs to treat chronic diseases without adverse effects is one of the major challenges for pharmaceutical industry [15, 16]. Among the strategies useful to discovering new drugs, the molecular modification is very promising strategy. Based on this 1, 2, 4-Triazine derivative of molecular docking studies was done against ligands [17].

Hence this study was designed to do synthesis and evaluation of 1, 2, 4–Triazin analogue of diclofenac as potential ligand to improve the dopamine level in the PD.

CHAPTER -2

LITERATURE REVIEW

CHAPTER - 2

LITERATURE REVIEW

2.1 NEURODEGENERATIVE DISEASE

Neurodegenerative diseases are commonly defined as disorders with selective loss of neurons and distinct involvement of functional system defining clinical presentation[18]. “Neurodegeneration” Etymologically, the word is composed of the prefix “neuro-,” which designates nerve cells (i.e., neurons), and “degeneration,” which refers to, the presence of tissues or organs, losing structure or function [19].

Neurodegenerative disorders (ND) are characterized by progressive dysfunction and loss of neurons and synapses in selected (vulnerable) areas of the nervous system. The major basic processes inducing ND are considered as multifactorial such as genetic, environmental, and endogenous factors related to aging [20].

In ND due to slow progressive loss of neurons in the central nervous system (CNS), affecting brain functions such as movement disorders, cognitive impairment, dysautonomia and memory [21]. Examples for NDs include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and multiple system atrophy (MSA) [22].

2.1.1 HISTORY

J. F. Bray was the first author to include the adjective neurodegenerative in the title of a medical article. It was published in 1965 in a British journal and was followed in 1968 by an article of D. A. Drachman in the American journal. Bray described a child with abnormal hair and “spastic dementia” and Drachman reported four cases of progressive ophthalmoplegia and CNS disorders.

The authors provided no definition for the term neurodegenerative but they grouped the so-called neurodegenerative disorders “for convenience” under three headings: hereditary neuropathies, hereditary spinocerebellar degenerations, and motor neuron disease. Neurodegenerative diseases are predominantly genetic disorders [23].

According to Prusiner, neurodegenerative diseases are progressive nervous system disorders of protein processing. 25 years ago, there was little understanding

of the causes of neurodegeneration. He explains, “a brief list of more common disorders and a few less common maladies” comprised Prion diseases, dementias, Parkinson disease and related disorders, motor neuron disease, Huntington disease, and spinocerebellar atrophies.’

The Medical Research Council (MRC) states in a strategic review that neurodegenerative diseases are incurable and debilitating conditions that result in progressive deterioration or death of neuron cells. They include Parkinsonism diseases, Alzheimer’s disease and other dementias, Huntington disease, motor neurone disease, Creutzfeldt-Jacob Disease, and multiple sclerosis [23].

However, demographically all over the world approximately 7.5 percent belong to the 60+ generation billion peoples who are battling from the neurodegenerative problems. In India is the home of more than eighty million people older than sixty years as per the 2011 Census.

According to World Health Organization (WHO), the rate of growth will be the highest (around 336%) in India, followed by China, South Asia, and western Pacific regions, 235-393% in Latin America and Africa, and the lowest (100%) in developed regions. The number of persons with dementia double every five years of age and so. India will have one of the largest numbers of elders with this degenerative problem [24].

2.1.2 ETIOLOGY AND PATHOLOGY

The etiology and pathogenesis of these devastating diseases remain largely unknown; however, the study of the neurochemistry and synaptic transmission. The different neurotransmitter change has helped to characterize involvement of specific subpopulations of neurons. The pattern of selective vulnerability of neurons provides important clues to pathogenesis and genetic regulation of neuronal development, and endogenous and exogenous neurotoxins [25]. Neurodegenerative disorders (NDD) constitute a set of pathological conditions originating from slow progressive and irreversible dysfunction and loss of neurons and synapses in selected areas of the nervous system [26]. Cellular and sub cellular pathology, this means whether neurons or glial cells (either or both astro- and oligodendroglia), including which compartment of the cells, show pathological protein deposits; or whether these are found extracellularly [27].

2.1.3 CLASSIFICATION

A nosological classification of neurodegenerative diseases is based on clinical presentation, Anatomical Involvement of Neuronal Loss Underlying Clinical Symptomatology and Neuropathological-Biochemical Classification

2.1.3.1 Anatomical Involvement of Neuronal Loss Underlying Clinical Symptomatology

Cognitive decline, dementia, and alterations in high-order brain functions are associated with involvement of the entorhinal cortex, hippocampus, limbic system and neocortical areas. A subtype of dementia is frontotemporal dementia (FTD), which is associated with degeneration of the frontal and temporal lobes (frontotemporal lobar degeneration, FTLD).

In movement disorders the basal ganglia, thalamus, brainstem nuclei, cerebellar cortex and nuclei, motor cortical areas and lower motor neurons of the spinal cord are involved. Combinations of these symptoms are observed in some diseases (i.e., prion diseases) early during the clinical course and in many disorders during the Progression [28].

2.1.3.2 Neuropathological-Biochemical Classification

This focuses primarily on the evaluation of the anatomical distribution of neuronal loss, and additional histological features (e.g., vascular lesions or spongiform change of the neuropil), and the distinction of intracellular and extracellular protein accumulations, which are analyzed by immunohistochemistry complemented by biochemistry. There are some aspects, which need to be clarified to understand the neuropathological approach.

Firstly, not all protein-deposits visible by immunohistochemical methods show the amyloid staining property (means that a particular structure shows applegreen birefringence under polarized light when stained with the Congo red dye) even though they are composed of proteins with highly ordered stacks of β - sheet-rich elements.

Secondly, for some proteins, synaptic location is mentioned; however, except in the case of prion disease, this is not respected for diagnostic classifications. It is important to distinguish the subcellular location of the intracellular deposits; whether

they are nuclear, cytoplasmic, or neuritic (axonal or dendrites), or in cellular processes (i.e., for astrocytes). For some diseases, only morphological criteria are used for subtyping, whereas for others biochemical modifications or even a gene polymorphism are also considered. For all neurodegenerative proteinpathies there are hereditary forms described [29].

2.1.4 MECHANISM OF ND

The mechanisms that cause neurodegeneration includes; 1) genetic mutations, 2) protein misfolding, 3) proteinopathy, 4) neuronal cell membrane damage, 5) mitochondrial dysfunction, 6) axonal transport defects and 7) programmed cell death or apoptosis. The based on clinical presentations and epidemiological studies have also incriminated age, environmental toxins and genetics as causation factors [30].

2.1.5 SYMPTOMS

These are determined by the anatomical region showing neuronal dysfunction or loss and do not necessarily reflect the molecular changes in the background.

Accordingly, in association with a neurodegenerative syndrome one can define anatomical, cellular and protein vulnerability. Genetic alterations can also lead to these alterations or influence the susceptibility to develop these diseases.

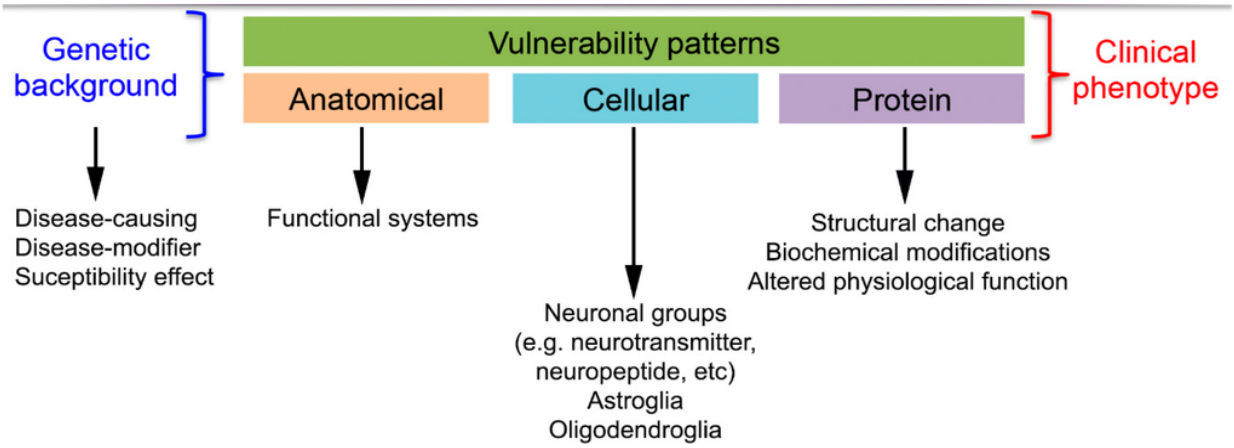


Figure No 1. Symptoms of Neurodegenerative Disorder

2.2 PARKINSON'S DISEASES

Parkinson's disease (PD) is a common, slowly progressive neurodegenerative disorder without a cure and affecting a wide range of motor and non-motor functions, and leading to marked disability in its later stages [31]. It is mainly characterized by diminished facial expression, stooped posture, slowness of voluntary movement, festinating gait (progressively short- ended, accelerated steps), rigidity and a pill-

rolling tremor [32]. Clinically, PD is associated with resting tremor (trembling), postural instability, rigidity, bradykinesia (slowness and minimal movement) and an impairment of postural balance leading to disturbance of gait and falling [33].

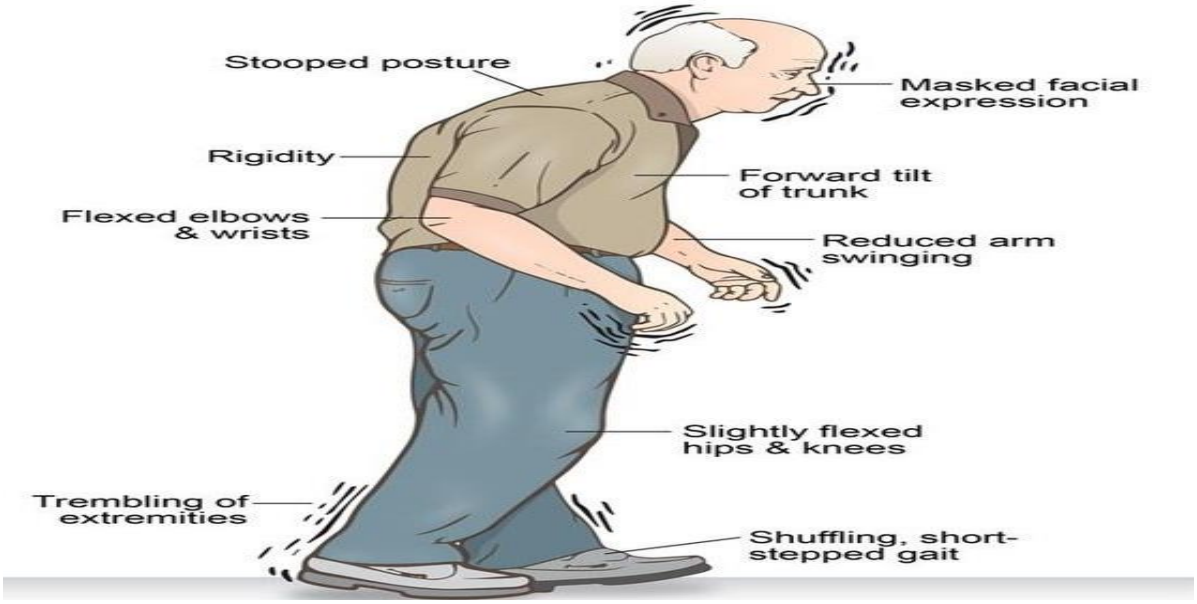


Figure No 2. Parkinson's symptoms

2.2.1 CLINICAL FEATURES

Motor Symptoms: From the motor standpoint PD is characterized by a clinical syndrome universally known as Parkinson's, which includes four cardinal features: bradykinesia, rest tremor, rigidity, and postural and gait impairment. One should bear in mind that these are not always observed in every patient, at least in a given time frame.

2.2.1.1 Bradykinesia refers to slowness of movements with a progressive loss of amplitude or speed during attempted rapid alternating movements of body segments. some repetitive movements as quickly and widely as possible, namely, opening and closing the hand, tapping thumb and index fingers, or tapping the foot on the ground. It can also be searched for globally by observing the spontaneous movements while sitting, standing up from a chair, or walking. Other clinical displays of bradykinesia are hypomimia (decreased facial expression and eye blinking, termed "poker face" in milder stages, hypophonia, micrographia (progressively smaller handwriting), and difficulty swallowing.

2.2.1.2 Rest tremor is the most common and easily recognized symptom of PD. Tremors are unilateral, occur at a frequency between 4 and 6 Hz, and almost always are prominent in the distal part of an extremity. Hand tremors are described as

supination–pronation (‘‘pill-rolling’’) tremors that spread from one hand to the other. Rest tremor with PD can also involve the lips, chin, jaw and legs but, unlike essential tremor, rarely involves the neck/head or voice. Parkinson’s related postural tremor (‘‘re-emergent tremor’’) is differentiated from essential tremor in that the appearance of tremor is often delayed after assumes an out stretched horizontal position.

2.2.1.3 Rigidity is characterized by increased resistance, usually accompanied by the ‘‘cogwheel’’ phenomenon, particularly when associated with an underlying tremor, present throughout the range of passive movement of a limb (flexion, extension or rotation about a joint). It may occur proximally (eg, neck, shoulders, hips) and distally (eg, wrists, ankles).

2.2.1.4 Postural and Parkinson’s gait impairment is slow, occurs on a narrow base, and is characterized by short shuffling steps, which gives the observer the impression of center of the gravity. There is decreased arm swing, turning around is slow and performed with multiple small steps [34. 35].

2.2.1.5 Non motor Symptoms and the Premotor Phase of PD; PD has been traditionally regarded as a motor disorder, perhaps because the original account of the clinical features emphasized these symptoms, while failing to recognize the important nonmotor aspects of the disease. In addition, motor symptoms often meet the eye straight away, even for untrained observers. However, in recent years there has been an increasing interest in nonmotor symptoms of PD Because of long-term pathological progression, some of these non motor features may be present before any of the classical motor signs are noticeable, in early disease stages, such as hyposmia, rapid eye movement (REM) behavior disorder, constipation, and depression. On the other hand, features like dementia and hallucinations occur late in the course of disease, which is useful for distinguishing PD from other disorders [36].

2.2.2 HISTORY

Parkinson’s disease was first medically described as a neurological syndrome by James Parkinson in 1817, though fragments of Parkinsonism can be found in earlier descriptions. Charcot described in full detail the arthritic changes, dysautonomia, and pain that can accompany Parkinson’s disease. Charcot was also the first to suggest the use of the term “Parkinson’s disease” rejecting the earlier designation of paralysis agitans or shaking palsy, because he recognized that Parkinson’s disease patients are not markedly weak and do not necessarily have

tremor. Gowers offered one of the most memorable similes regarding Parkinsonian tremor. Further clinical descriptions and studies of the pathologic changes related to Parkinson's disease were predominantly reported by the French neurologic school. Richer and Meige (1895) provided clinical and morphologic details of the progressive stages of Parkinsonian disability, and the former provided drawings and statues that remain among the most important pictorial documents related to Parkinson's disease [37].

2.2.3 ETIOLOGY AND PATHOGENESIS

The view of etiological factors in PD has changed remarkably from one of a purely sporadic basis to the view that both environmental and genetic factors contribute to the onset of the illness to a point now where increasingly genetic predisposition must be seen as a major contributor to the underlying cause. The specific etiology of PD is not known [38.]

2.2.3.1 ENVIRONMENTAL FACTORS: It may increase the risk of developing PD. These include exposure to well water, pesticides, herbicides, and industrial chemicals, wood pulp mills, farming, and living in a rural environment. A number of exogenous toxins have been associated with the development of parkinsonism, including trace metals, cyanide, lacquer thinner, organic solvents, carbon monoxide, and carbon disulfide. There has also been interest in the possible role of endogenous toxins such as tetrahydroisoquinolines and beta-carbolines. However, no specific toxin has been found in the brain of PD patients, and in many instances the Parkinson's seen in association with toxins is not that of typical Lewy body PD.

2.2.3.2 GENETIC FACTORS: Leads to decreasing the risk of developing PD can also provide valuable clues to its etiology. The evidence for cigarette smoking and caffeine intake in reducing risk appears clear, but there is still uncertainty over the role of others, for example, exercise, anti-inflammatories, anti-hypertensives (most notably calcium antagonists), and anti-lipidaemics [38, 39].

2.2.3.3 ENERGY, METABOLISM, AND AGING: The excitotoxic hypothesis provides a link between patterns of neuronal injury, the effects of aging, and observations on the metabolic capacities of neurons. Since the ability of Mg2+ to block the NMDA receptor-channel is dependent on the membrane potential, disturbances that impair the metabolic capacity of neurons will tend to relieve Mg2+ blockade and predispose

to excitotoxic injury. The capacity of neurons for oxidative metabolism declines progressively with age, perhaps in part because of a progressive accumulation of mutations in the mitochondrial genome. Patients with PD exhibit several defects in energy metabolism that are even greater than expected for their age, most notably a reduction in the function of complex I of the mitochondrial electron transport chain. Addition evidence for the role of metabolic defects in the etiology of neural degeneration comes from the study of patients who inadvertently self- administered MPTP induces studies have shown that a metabolite of MPTP induces degeneration of neurons similar to that observed in idiopathic PD and that its mechanism of action appears to be related to an ability to impair mitochondrial energy metabolism in dopaminergic neurons.

2.2.3.4 OXIDATIVE STRESS: Signs of oxidative stress are abundant in the substantia nigra of patients with PD. Mitochondrial complex I activity is depressed. Levels of intrinsic antioxidants, such as glutathione, are reduced, while oxidized products of proteins, lipids, and DNA increase significantly. Increasing levels of oxidative stress can eventually lead to apoptosis through the intrinsic (or "mitochondrial") PCD pathway due to cytoplasmic release of cytochrome c, which is proapoptotic, from dysfunctional mitochondria.

2.2.3.5 INFLAMMATION: Local inflammation is readily apparent at sites of neuron loss in both PD. Most of the inflammatory cells at these sites are activated microglia, although lesser numbers of reactive astrocytes are seen as well. While the astrocytes are suspected of playing an overall protective role in PD by such mechanisms as sequestration and metabolization of DA, glutathione-mediated scavenging of ROS and production of glial-derived neurotrophic factor (GDNF), the microglia are believed instead to facilitate the neurodegenerative process in PD [40, 41].

2.2.4 EPIDEMIOLOGY

PD is a prevalence of approximately 1% of the population above 60 years, which reaches a prevalence of nearly 4% by age 50. PD is the second most common neurodegenerative disorder. The mean age of PD diagnosis is in the seventh decade of life, but due to PD's insidious nature, the onset of symptoms may precede clinical recognition by many years. The annual incidence per 100,000 inhabitants ranges from less than 10 to more than 20. Incidence studies may be affected by under-

diagnosing of PD, especially among the most elderly. This study reported an annual incidence of 18.8 cases per 100,000 and a mean age of onset at 70.6 years, slightly above the mean age of onset that we reported from Norway. Early-onset of PD is rare in population-based studies. PD represents a frequent cause of morbidity that affects 1–2 per 1000 of the population at any time, clearly most often in the older age groups. It affects men slightly more frequently than women. There may be an increase in occurrence of the disease that cannot be explained by demographic changes of the population alone. It is generally accepted that the prevalence of the disease range from 1 to 2 per 1000 in unselected populations and that the disease. Most of the increase could be attributed to the general increase in age of the population. However, recently published data support an increase in risk of PD, especially in men. It was hypothesized that the increase in risk of PD could be related to the dramatic changes in smoking behavior that has been taken place during the last part of the twentieth century. Increased occurrence of PD has also been related to increase in traffic-related air pollution [42, 43].

2.2.5 DIAGNOSTIC CRITERIA

Having established that the patient has Parkinson's, the (movement disorder society) MDS-PD criteria will be applied to determine whether the patient meets criteria for PD as the cause of this Parkinson's.

Diagnosis of clinically established PD requires:

1. Absence of absolute exclusion criteria
2. At least two supportive criteria
3. No red flags

Diagnosis of clinically probable PD can be made in:

1. Absence of absolute exclusion criteria
2. Presence of red flags counterbalanced by supportive criteria, ie, if one red flag is present there must also be at least one supportive criterion; if two red flags, at least two supportive criteria are needed. If there are more than two red flags, clinically probable PD cannot be diagnosed [44].

2.2.6 DIFFERENTIAL DIAGNOSES

Once PD is identified, it is important to consider other conditions in the differential diagnosis. As mentioned above, diagnosis of PD is often found to be inaccurate at specialty clinics or at postmortem review. Presence of “red-flag” clinical features response to levodopa treatment should alert family physicians to the possibility of an alternative diagnosis. The main conditions to consider are drug-induced Parkinson’s, vascular Parkinson’s, progressive supranuclearpalsy, multiple system atrophy, and DLB. The diagnosis is normally confirmed by a neurologist. Neuroimaging does not have a big role [45].

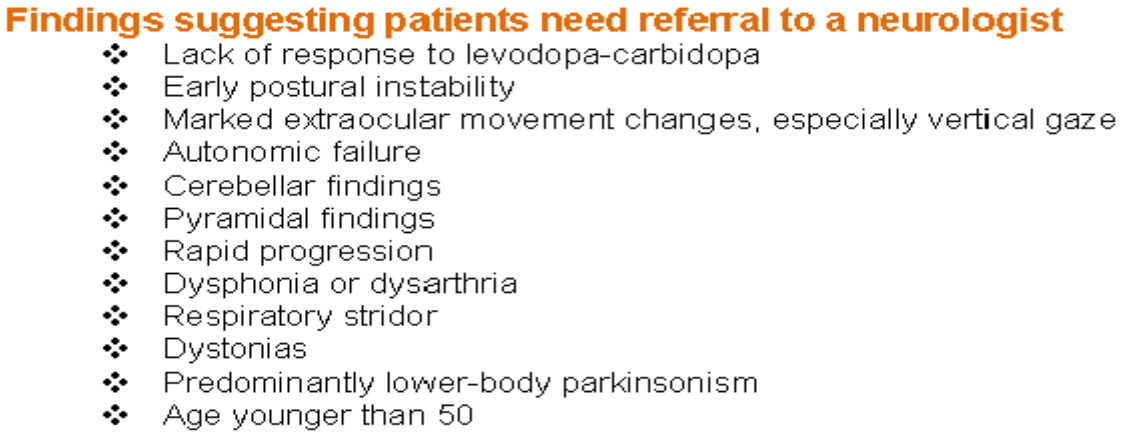


Figure No 3. Findings suggesting patients need to aneurologist

2.2.7 TREATMENT OF PD

The treatment of PD over the past half century, but levodopa remains the most potent drug for controlling PD symptom. Prior to instituting medical therapy, a correct diagnosis of PD must be established and the level of impairment (motor, sensory, autonomic and mental) determined. Each therapy is to be individualized, and diverse drugs other than levodopa are presently available. Among these are the dopamine agonists (DA), catechol-o-methyl-transferase (COMT) inhibitors and non dopaminergic agents [46].

2.2.7.1 LEVODOPA

The introduction of dihydroxyphenylalanine (levodopa) to the treatment of PD was a major scientific and clinical breakthrough in the treatment of this devastating disease. The enzyme involved in the transformation of levodopa toDA, ie, l-amino acid decarboxylase (L-AAD, initially calleddopa decarboxylase) is widespread in the body, with high concentrations in the liver. it is essential that levodopa be converted into DA in the brain, and so the L-AAD inhibitor should not cross the BBB. The inhibition of peripheral L-AAD has another result, which was initially unappreciated: it prolongs the biological half-life of levodopa (and therefore also of DA in the brain). The pharmacokinetic and pharmacodynamic changes that take place as the disease progresses may be major contributors. It has also been speculated that the complications may derive, at least in part, from the toxic effects of levodopa or DA oxidative metabolites.It quickly became clear also that, of the two dopa isomers, only the levorotatory stereoisomer, levodopa, produced therapeutic benefits, and chemical means to separate the two isomers were developed. In practice, only levodopa is now used in the treatment of PD, resulting in an improved safety profile.

2.2.7.2 COMT INHIBITORS

Catechol-O -methyltransferase (COMT) is a ubiquitous enzyme that breaks down levodopa before it can be converted to DA, as well as DA itself. COMT inhibitors prolong the availability of a single dose of levodopa, without delaying the onset of its effects, frequently reducing the total amount of levodopa needed. However, COMT treatment in the earlier stages of PD may also be worthwhile by preventing or delaying motor complications. COMT inhibition as a new treatment strategy for PD has been recently comprehensively reviewed [46].

2.2.7.3 DA AGONISTS

Dopamine agonists (DA) exert their pharmacologic effect by directly activating DA receptors, bypassing the presynaptic synthesis of DA. In order to delay or prevent levodopa-induced complications many parkinsonologists recommend using DA agonists as the initial or early form of dopaminergic therapy. Dopamine agonists (DA) exert their pharmacologic effect by directly activating DA receptors, bypassing the presynaptic synthesis of DA. D2 receptors is important in mediating the

beneficial anti parkinsonian effects of DA agonists, but concurrent D1 and D2 stimulation is required to produce optimal physiological and behavioral effects.

2.2.7.4 NONDOPAMINERGIC THERAPY

In addition to the dopaminergic drugs, nondopaminergic drugs, such as the anticholinergics and amantadine, may provide satisfactory symptomatic relief in early phases of anti-PD therapy. A novel antidepressant that enhances noradrenergic and serotonergic transmission and acts as a presynaptic alpha-2, 5HT2, and 5HT3 receptor antagonist has been reported to improve rest tremor and levodopa-induced dyskinesia [47].

2.2.3 NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs in modern medicine. NSAIDs are very effective in the alleviation of pain, fever and inflammation, and millions of patients worldwide have found relief in their use since the discovery of the soothing properties of willow bark more than 3,500 years ago. NSAID use is however associated with several serious treatment side effects, with considerable associated morbidity and mortality. Many of these side effects may be prevented by careful consideration of the patient's risk factors and by subsequent implementation of preventive strategies [48]. It is generally thought that one of their main mechanisms of action is the inhibition of cyclo-oxygenase (COX), the enzyme responsible for biosynthesing the prostaglandins and thromboxane [49].

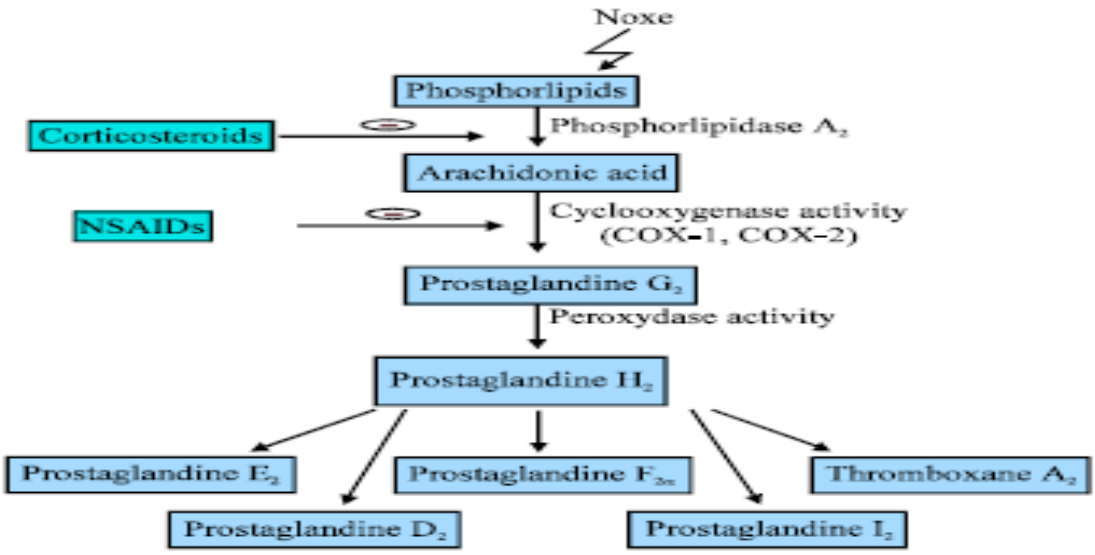


Figure No 4. NSAIDs mechanism of action

2.3.1 RISK FACTORS FOR GASTROINTESTINAL COMPLICATIONS OF NSAIDs

Dyspeptic symptoms are not a trustworthy warming sign; because of that it is important to identify factors that increase the risk of GI events in NSAIDs users. Among them, the two main are prior history of complicated ulcers, the most important one, and age. Older age is the most common in NSAIDs users and those age> 70 years are considered to carry a risk similar to those with history of peptic ulcer [50].

2.4 1, 2, 4-Triazin derivative on PD

Potent, ligand efficient, selective, and orally efficacious 1, 2, 4-Triazin derivatives have been identified using structure based drug design approaches as antagonists of the adenosine A2A receptor. Structure of ligands to a G protein – coupled receptor can be used to direct optimization of novel, low molecular weight into high potent and selective lead compounds. In this 1, 2, 4-Triazin derivative desirable physicochemical and drug like properties, including high oral bioavailability and very potent in vivo efficacy and preclinical candidate for the potential treatment of PD.

2.5 DOCKING

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Preferred orientation helps to predict the strength of association of or binding affinity between two molecules. The associations with biological molecules such as proteins, nucleic acids, carbohydrates and lipids play an important role in signal transduction i.e. agonism or antagonism. So docking is a useful tool for predicting both the strength and type of signal produced.

2.6 MOLECULAR DOCKING

Molecular docking may be defined as an optimization program, which would describe the ‘best-fit’ orientation of a ligand that binds to a particular protein of interest. The focus of molecular docking is to computationally stimulate the molecular recognition process. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized. Docking

produces plausible candidate structures. These candidates must be ranked by using scoring functions and to identify structures that are most likely to occur in nature. Calculation of ligand-protein interaction by a scoring function that includes terms and equations that describe the intermolecular energies [51].

CHAPTER - 3

DRUG PROFILE

CHAPTER - 3

DRUG PROFILE

3.1 DICLOFENAC

Diclofenac is a proven, commonly prescribed non steroidal anti-inflammatory drug (NSAID) that has analgesic, anti-inflammatory, and antipyretic properties, and has been shown to be effective in treating a variety of acute and chronic pain and inflammatory conditions.

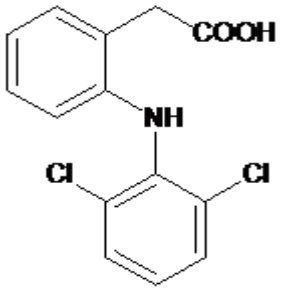


Figure No 5. Structure of Diclofenac

IUPAC Name **:** 2-[2-(2, 6-dichloroanilino) phenyl] acetic acid

Molecular Formula: $C_{14}H_{11}Cl_2NO_2$

Molecular weight **:** 296.147g/mol

Description **:** It is a white or almost white crystalline powder

Melting Point **:** 156-158 º C

Solubility **:** It is partially insoluble in water, very slightly soluble in ethanol and methyl alcohol

Category **:** Non steroidal Anti inflammatory drug

3.1.1 MECHANISM OF ACTION

Diclofenac belongs to a group of NSAIDs that inhibit both COX-1 and COX-2 enzymes. The binding of NSAIDs to COX isozymes inhibits the synthesis of prostanoids (i.e., prostaglandin [PG]-E_2, PGD_2, PGF_2, prostacyclin [PGI_2], and thromboxane [TX] A_2) [52]. PGE_2 is the dominant prostanoid produced in inflammation, and the inhibition of its synthesis by NSAIDs is believed to be the main mechanism of the potent analgesic and anti-inflammatory properties of these agents.

Although diclofenac is commonly referred to as a traditional NSAID in the published literature, these assays have demonstrated that it has a higher selectivity for COX-2 than for COX-1, in contrast with most traditional NSAIDs. The degree of COX-2 selectivity demonstrated for diclofenac is comparable to that of celecoxib. diclofenac is more potent in inhibiting COX-2 than COX-1 isoenzymes. However, the estimated IC_{50} (concentration causing 50 % inhibition of activity) values for COX-1 and COX-2 of different COX inhibitors have been shown to vary between models and selectivity is dose dependent in some cases [53].

3.1.2 DOSE

Diclofenac was given orally or by intramuscular injection in doses ranging from 50 to 75 mg daily, or up to 150 mg per day for longer-term use. There is no evidence that the dosage of diclofenac needs to be modified in patients with the mild renal impairment, but as with other NSAIDs caution should be exercised.

3.1.3 PHARMACOKINETICS

Absorption of diclofenac is generally rapid and directly proportional to the dose. The rate of diclofenac absorption may vary depending on the salt form, pharmaceutical composition, and timing of administration in relation to food intake. The absorption of diclofenac can be inconsistent, with variable maximum plasma concentration (C_{max}) and time to C_{max} (t_{max}), as well as the presence of late or secondary plasma peaks in plots of diclofenac concentration versus time. It has been proposed that these inconsistence’s in diclofenac absorption arise due to individual

subject differences in GI pH, partial precipitation of the dose in the acidic conditions in the stomach, variable timing in gastric emptying, and enterohepatic circulation [54].

Acidic NSAIDs are highly bound to plasma proteins, mainly albumin. Diclofenac penetrates into the synovial fluid where the concentrations reach approximately 60% of those in plasma. The volume of distribution is approximately 30L.

Approximately 60 % of the intact diclofenac reaches the systemic circulation due to first-pass metabolism. The main metabolite, 4′-hydroxydiclofenac, is known to retain weak anti-inflammatory and analgesic activities. Following biotransformation to glucoroconjugated and sulphate metabolites, diclofenac is excreted in the urine.

Because of its short biological half-life (~2 h) and fast elimination rate (mean elimination half-life 1.2–1.8 h), frequent administration of diclofenac is usually necessary to maintain its therapeutic concentration, which could in turn increase the risk for adverse events [55].

3.1.4 PHARMACODYNAMICS

Diclofenac directly blocks the PGE2 secretion at the site of inflammation by inhibiting IL-Beta & TNF in the inflammatory cells (intracellular Action). Diclofenac has been demonstrated to inhibit cyclooxygenase (COX) activity and to suppress the PGE2 production by inflammatory cells, which are likely to be a primary source of PGE2. Inflammatory cells release IL-1 and TNF, which produce the PGE2 by induction of the COX-2. diclofenac and 4'-hydroxydiclofenac penetrate the inflammatory cells like polymorphunuclears, monocytes and rheumatoid synovial cells and get hydrolysed to the active metabolites of diclofenac and 4'-hydroxyaceclofenac which inhibits IL-1 and TNF released by the inflammatory cells and therefore suppress production of PGE2 at the site of inflammation.

3.1.5 DICLOFENAC SAFETY CONSIDERATIONS

3.1.5.1 GASTROINTESTINAL CONCERNS

From their first description, traditional NSAIDs have been associated with GI side effects including life-threatening GI hemorrhage. Diclofenac, as discussed

earlier, has more specificity to COX-2 than COX-1, and is associated with a relatively low level of GI toxicity compared with the other NSAIDs. Because of purported causal relationship between COX-1 inhibition and GI adverse effects, the COX-2-specific NSAIDs were developed to lower the GI risk [56].

3.1.5.2 CARDIOVASCULAR CONSIDERATIONS

The COX-2-selective inhibitors were designed to limit the known GI adverse events associated with the drug class. However, with the increased use of COX-2 inhibitors, further analysis of the data, and additional reports, it became clear that COX-2-selective inhibitors may be associated with an increase in cardiovascular risk. The increased cardiovascular risk associated with COX- 2-selective inhibitors has been suggested to be due to a disruption in the normal balance between the prothrombotic activity of thromboxane A2 (TXA2) (derived from COX-1 activity) and the inhibition of platelet aggregation by PGI2 (derived from COX-2 activity). In other words, bleeding or excessive platelet aggregation is hypothesized to occur if this balance is tipped toward relatively higher activity of PGI2 or TXA2, respectively. For example, it is thought that excessive bleeding associated with traditional NSAIDs is due to inhibition of constitutively expressed COX-1 activity (and subsequent decrease in platelet aggregation), via a decrease in TXA2, the major product of platelet COX-1 [57].

3.1.5.3 RENAL COMPLICATIONS

In addition to GI and cardiovascular concerns, the prolonged use of NSAIDs has been linked to renal injury and toxicity. Although reports have demonstrated that selective COX-2 inhibitors have effects on renal function similar to those observed with nonselective NSAIDs. Acute treatment with therapeutic doses of diclofenac has not been associated with significantly impaired renal function in healthy adults or post surgical patients. However, the results from a long-term study (46 months) have indicated that prolonged treatment with diclofenac can be associated with decreases in creatinine clearance, a predictor in renal dysfunction. Importantly, a time course evaluation of decreased clearance revealed that the highest frequency of this change occurred in early visits during the treatment period [58, 59].

3.1.5.4 HEPATIC ADVERSE EFFECTS

As with antihypertensives, antimicrobials, and immunosuppressants, NSAIDs can cause drug-induced liver injury. It has been reported that diclofenac causes elevations of transaminase levels more commonly than other NSAIDs. Yet, a review of in vitro and in vivo animal studies did not indicate that diclofenac is hepatotoxic. While anecdotal reports rarely associate diclofenac with hospitalizations due to liver toxicity and acute liver failure, a systemic review of over 60 randomized, controlled clinical trials did not associate an increase in clinical liver events with diclofenac compared with other NSAIDs. Nonetheless, it is recommended that physicians educate their patients on the warning signs of hepatotoxicity, and to minimize potential risk by treating patients with NSAIDs with the lowest effective dose for the shortest duration. Further, as with other NSAIDs, transaminase levels should be measured periodically in patients receiving diclofenac as severe hepatic reactions can occur at any time during treatment [60].

3.1.5.5 ADVERSE EVENTS ASSOCIATED WITH TOPICAL FORMULATIONS

Topical NSAIDs have been postulated to be as efficacious as oral treatment without GI, hepatic and renal side effect complications. As the risk of these events increases with age, topical NSAIDs are an attractive option for the treatment of pain relief. Overall, reports in the literature regarding adverse events for topical diclofenac are limited and details regarding safety are often generalized. Typically reported adverse events for topical diclofenac include localized skin reactions including rash, itching, or burning. In comparison to oral treatment, topical diclofenac is generally well tolerated with a lower incidence of systemic adverse events including GI complaints. However, with a limited number of reports summarizing safety of topical diclofenac, additional studies are necessary to ascertain any long-term safety risk of topical diclofenac specifically or for any safety comparisons with oral formulations. Currently, topical formulations of diclofenac carry the same warnings for an increased risk of cardiovascular and GI events, as well as renal and hepatic adverse effects that are carried by oral formulations of diclofenac and other NSAIDs [61].

CHAPTER-4

AIM AND OBJECTIVE

CHAPTER - 4

AIM AND OBJECTIVES

Parkinson's disease (PD) is a common neurodegenerative disorder, which is characterized by slowly progressive loss of dopaminergic neurons associated with substantial morbidity and mortality and the number of persons affected is expected to increase dramatically in coming years.

NSAIDs medications like aspirin, ibuprofen, naproxen, Diclofenac, Mefenamic acid, Indomethacin Piroxicam and some selective COX-II inhibitors i.e., Celecoxib are one of the commonly used medication for the treatment of Parkinson's. Among this Diclofenac is the most preference drug.

Several previous studies have suggested that Diclofenac may elicit appreciable GI irritation, bleeding and ulceration produced. Synthetic approaches based upon chemical modification of Diclofenac have been taken with the aim of improving safety profile and in turn therapeutic window. Carboxylic group is a major reason for the GI toxicity of Diclofenac, so Structural replacement of carboxyl group may reduce the GI toxicity. Previous study reported that 1, 2, 4-Triazin derivative may possess appropriate Anti Parkinson's action.

Based on that aim of this study is replacement of carboxylic group with 2-Chloroacetamide to produce the 1, 2, 4 –Triazin derivative of Diclofenac as potential ligand for PD

Objectives of this study is

- ✓ Synthesis of 1, 2, 4-Triazin derivative of Diclofenac by Replacing the Carboxylic group with 2-Chloroacetamide.
- ✓ Molecular docking studies of 1, 2, 4–Triazin derivative of Diclofenac against PD targets.
- ✓ Increases the dopamine level against chlorpromazine induced PD rat

CHAPTER- 5

PLAN OF WORK

CHAPTER-5

PLAN OF WORK

1. **Selection and synthetic scheme of the Diclofenac Analogue**

   a) Step I: To Hydrolysis of Diclofenac sodium to 2-[(2, 6-dichloroanilino) phenyl] acetic acid.

   b) Step II: To Synthesis of ethyl-[2-(2, 6-dichloroanilino) phenyl] acetate.

   c) Step III: To Synthesis of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide.

   d) Step IV: To Synthesis of 3-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 6-dihydro-1, 2, 4-triazin-5(2H)-one.

2. **Determination of intermediate synthesized compound a, b, c, and d by FT-IR.**
3. **Molecular docking studies**
4. **Acute oral toxicity studies (OECD 423 guidelines) of 1, 2, 4-Triazin Analogue of Diclofenac**
5. ***In vivo* Anti Parkinson's studies**

   5.1 Selection of animal

   5.2 Animal grouping

   5.3 Induction of chlorpromazine induced Parkinson's disease

   5.4 Physical evaluation

   5.4.1 Body weight

   5.4.2 Feed intake

   5.5 Behavioural assessment

   5.5.1 Evaluation of exploratory and locomotors activity by using Actophotometer Apparatus

   5.5.2 Evaluation of muscle coordination by using Rota Rod

   5.5.3 Evaluation of cognitive performance by using Morris Water Maze Apparatus

   5.5.4 Evaluation of catalepsy

   5.6 Biochemical estimation

5.6.1 Estimation of serum glutamate pyruvate (SGPT)

5.6.2 Estimation of glutamate oxaloacetate transaminase (SGOT)

5.6.3 Estimation of Alkaline phosphatase (ALP)

5.6.4 Estimation of Total bilirubin

5.6.5 Estimation of Urea

5.6.6 Estimation of Creatinine

5.7 Estimation of antioxidant enzyme levels in rat brain

5.7.1 Estimation of superoxide dismutase (SOD)

5.7.2 Estimation of reduced glutathione (GSH)

5.7.3 Estimation of nitrite

5.7.4 Estimation of protein

5.7.5 Estimation of lipid peroxidation products

5.7.6 Estimation of catalase

5.7.7 Estimation of brain glutamate level

5.8 Estimation of brain tissue extract neurotransmitters

5.7.8 Estimation of brain Ach levels

5.7.8 Estimation of dopamine assay

5.9 Determination of ulcerogenecity

5.10 Histopathological evaluation

6 Statistical analysis

CHAPTER-6

MATERIALS AND METHODS

CHAPTER - 6

MATERIAL AND METHODS

6.1 DRUGS AND CHEMICALS

All the chemicals used in this study were of analytical grade. The following chemicals were used for the experimental study. **(Table No. 1)**

Table No 1. Drugs and chemicals

S.No.	MATERIAL	SOURCE
1	Diclofenac	Novartis Pvt. Ltd, Hyderabad
2	Chlorpromazine	Suri pharma laboratories Ltd, Gujarat
3	Methanol	Loba chemie Pvt. Ltd, Mumbai
4	Sodium bicarbonate	Loba chemie Pvt. Ltd, Mumbai
5	Hydrazine Hydrate	Nice chemicals Pvt.Ltd., Kerala
6	Dimethylformamide	Nice chemicals Pvt.Ltd., Cochin
7	2-Chloroacetamide	Avra Synthesis Pvt.Ltd, Hyderabad
8	Conc. Sulphuric Acid 98%	Loba chemie Pvt. Ltd, Mumbai
9	Gum acacia	Loba chemie Pvt. Ltd, Mumbai
10	Chloroform	chemie Pvt. Ltd, Mumbai
11	Diethyl ether	Loba chemie Pvt. Ltd, Mumbai
12	Chloroform	chemie Pvt. Ltd, Mumbai
13	Absolute ethanol	Changshu hongsheng fine chemical co., Ltd, Jiangsu province

6.2 SELECTION AND SYNTHETIC SCHEME DICLOFENAC ANALOGUE [62].

6.2.1 Step I: Hydrolysis of Diclofenac sodium to 2-[(2, 6-dichloroanilino) phenyl] acetic acid.

Diclofenac sodium (0.101) was dissolved in ethanol (2.5mol). To this solution conc. H2SO4 was added dropwise to hydrolyse the salt to acid. The acid obtained was filtered, dried. Yield: 96.34%. **(Figure No. 6)**

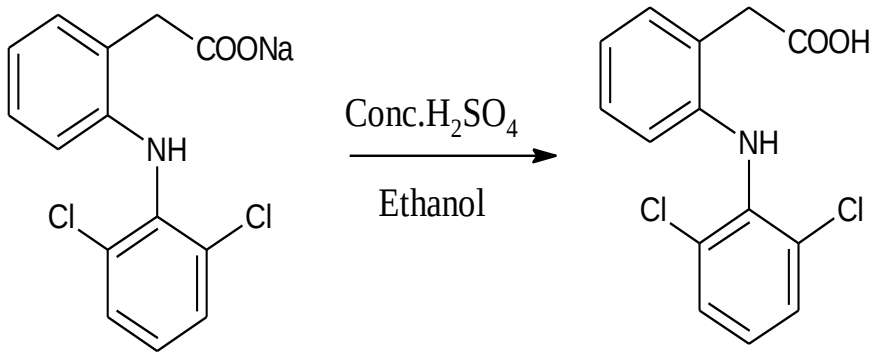


Figure No 6. Hydrolysis of Diclofenac sodium to 2-[(2, 6-dichloroanilino) phenyl] acetic acid.

6.2.2 Step II: Synthesis of ethyl-[2-(2, 6-dichloroanilino) phenyl] acetate.

0.05 mol of 2-[(2, 6-dichloroanilino) phenyl] acetic acid (Step II) was dissolved in absolute ethanol (10ml), conc. H2SO4 (1ml) was added and the reaction mixture was refluxed for 22 hrs. Reaction mixture gave on processing ethyl ester. The solid obtained was washed with 50 ml of sodium bicarbonate solution (10%) and recrystallized from methanol. Yield: 95.14%. **(Figure No. 7)**

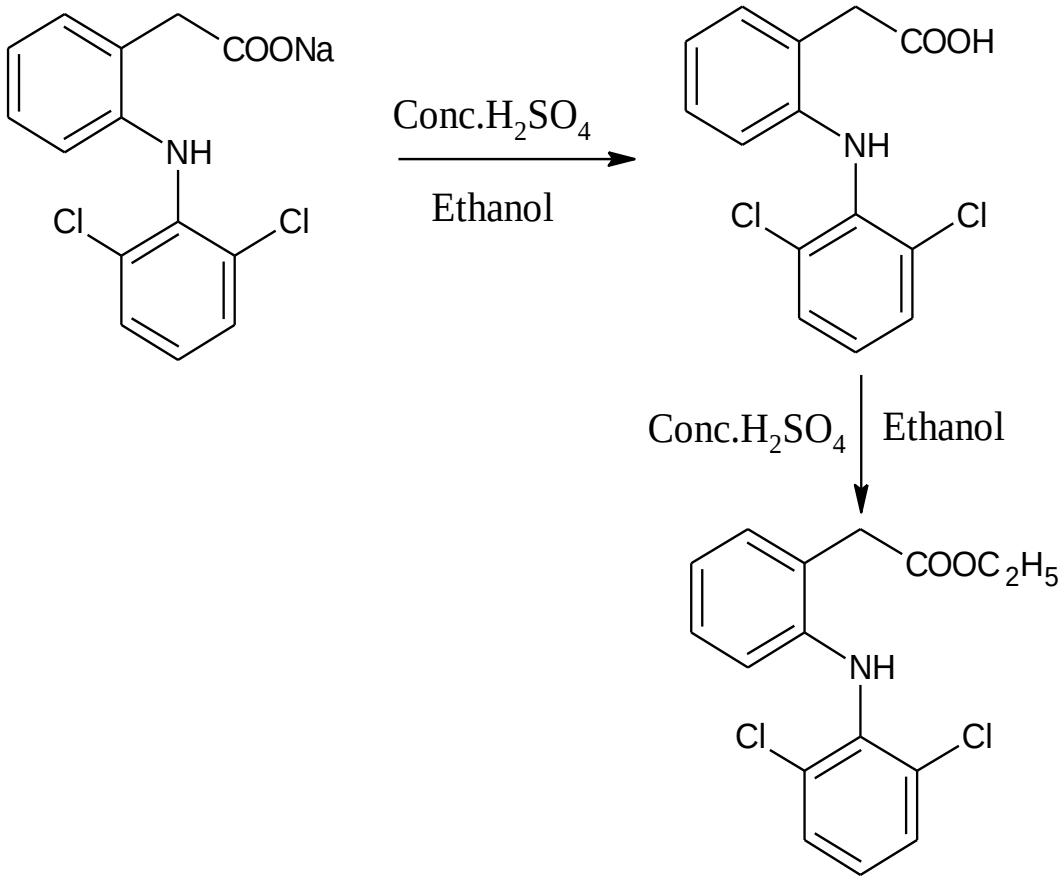


Figure No 7. Synthesis of ethyl-[2-(2, 6-dichloroanilino) phenyl] acetate

6.2.3 Step III: Synthesis of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide.

0.01 mol of Synthesis of ethyl-[2-(2, 6-dichloroanilino) phenyl] acetate (Step III) and hydrazine hydrate (0.02 mol) were refluxed in absolute ethanol (50ml) for 20hrs. The mixture was concentrated, cooled and poured in ice cold water. The solid thus precipitated out was filtered, dried and recrystallized from ethanol. Yield: 86.48%. (**Figure No. 8)**

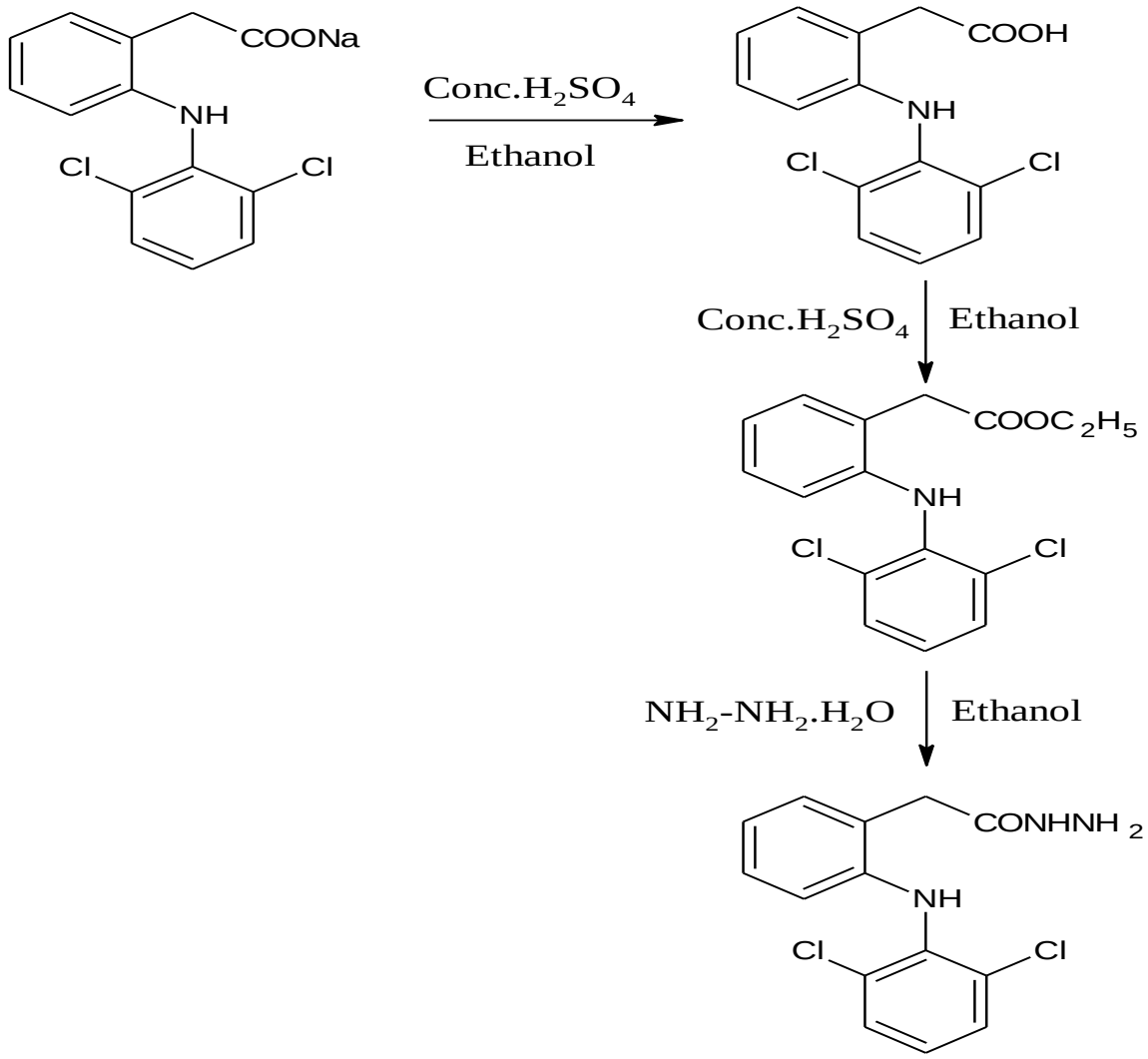


Figure No 8. Synthesis of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide.

6.2.4 Step IV: Synthesis of 3-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 6-dihydro -1, 2, 4-triazin-5(2H)-one

0.001 mol of Synthesis of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (Step IV), 2-chloroacetamide (0.001 mol) and dimethylformamide (80 ml) were added and the reaction mixture was refluxed for 30 hrs. It was then concentrated and cooled, whereupon the solid precipitated was filtered, washed with ethanol and recrystallized from DMF. Yield: 79.54%. (**Figure No. 9)**

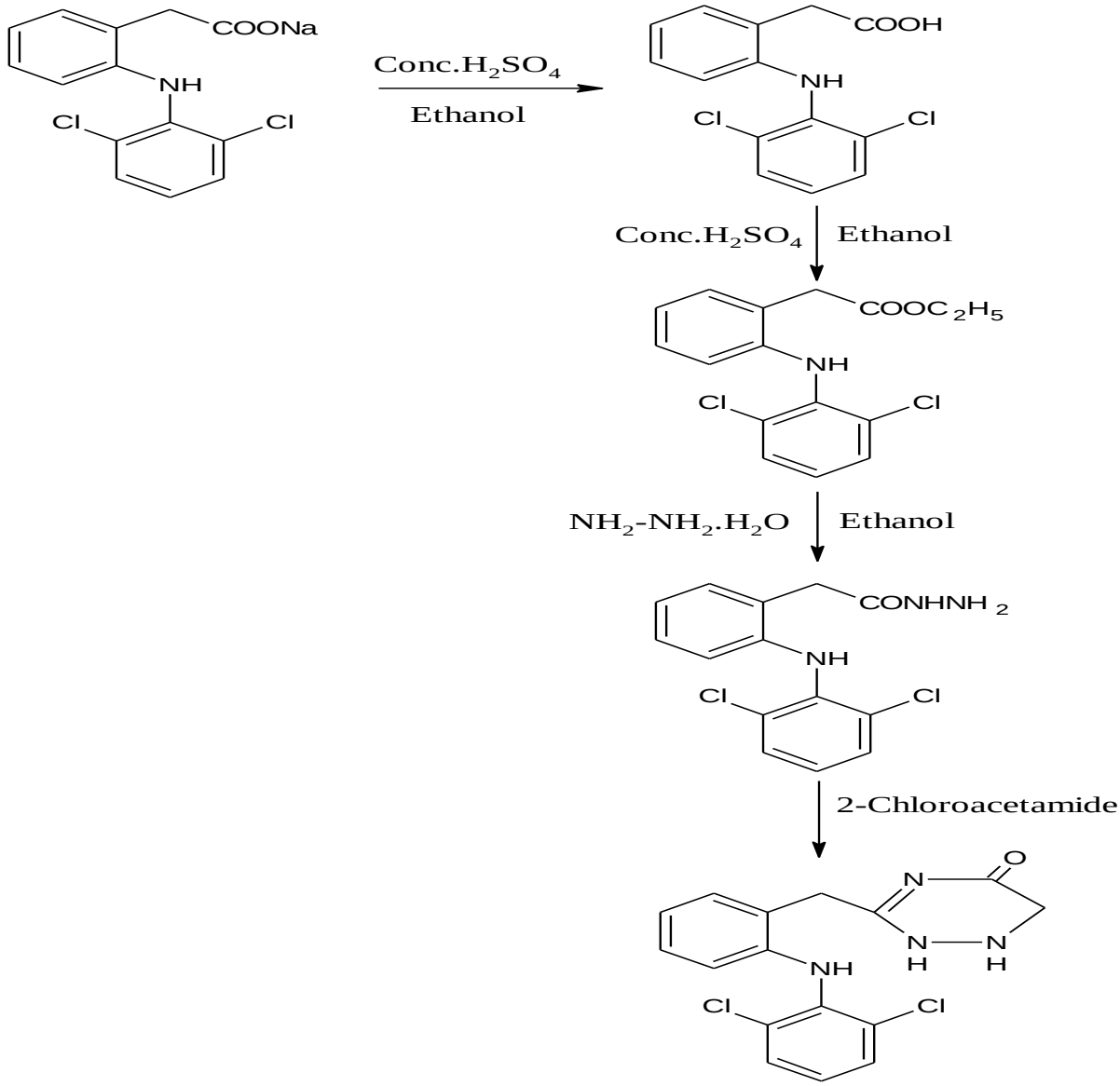


Figure No 9. Synthesis of 3-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 6-dihydro -1, 2, 4-triazin-5(2H)-one.

6.3 FT-IR SPECTRA

From the all analogue of Diclofenac scheme for the synthesis compounds were assigned on the basis of different spectral analysis. In IR spectral studies, the synthesized compound I, II, III, IV characteristic contains O-H Stretching, N-H Stretching, C-H aromatic stretching, C=O stretching, N-H Bending. 1, 2, 4-Triazine derivatives were synthesized in our laboratory. The all synthesized compounds a, b, c, and d was characterized by FTIR. The spectra data were satisfactory to prove the structure of compounds.

Specifications - Spectral Range: 7000 to 400 cm-1

- Resolution: 1cm-1, 2 cm-1, 4 cm-1, 8 cm-1
- Wave number Repeatability: Better than ±0.01cm-1
- Scanning Speed: 0.2~2.5 cm-1, automatically optimized for detector type or manually adjustable for specific applications
- Beam splitter: KBr
- Infrared Source: External, air-cooled, high efficiency Reflex Sphere module
- Detector: DTGS, MCT (optional)
- Data system: Compatible computer
- Output: Laser printer
- Software: FT-IR software contains all routines needed for basic spectrometer operations.

6.4 MOLECULAR DOCKING

6.4.1 Preparation of target

Docking is a method by which we can predict the preferred orientation, affinity and activity of a molecule to a targeted protein. The structure of the protein-ligand complexes for the Parkinsonism Disease associated targets were used for the molecular docking studies. They were downloaded from the research collaborator for structural bioinformatics (RCSB) Protein data bank (PDB; http://www.rcsb.org/pdb/home.do). For each crystal structure, the crystallographic water molecule were removed and edited by removing the hetero atoms [63, 64]. (**Table No. 2)**

Table No 2. 1, 2, 4-Triazin derivative of diclofenac against the Parkinson's enzyme targets

S.No.	NAME OF THE TARGET	PDB CODE
1	Dopamine receptor D3 protein	3PBL
2	Dopa decarboxylase (DDC)	1JS3
3	Adenosine A2 receptors (AA2AR)	3EML
4	P38 map kinase (MPAK)	2ZAZ
5	Monoamino oxidase-B (MAO-B)	2V5Z

6.4.2 Preparation of ligands

Structure of the 1, 2, 4-Triazine derivatives were retrieved. The structure of the ligands was drawn in ACD / ChemSketch freeware. Finally, compounds were saved in PDB format for further docking studies.

1, 2, 4-triazine derivative has desirable physicochemical and drug –like properties, including high oral bioavailability and very potent in vivo efficacy. Further

optimization of this 1, 2, 4 triazine derivative of targets has identification of a preclinical candidate for the potential treatment of Parkinson's disease. [65].

6.4.3 Docking simulation

Lamarckian genetic algorithm methodology was employed for docking simulations implemented in AutoDock k4. The standard docking procedure was used for a rigid protein and flexible ligand whose torsion angles were identified. A grid of 60, 60, and 60 points in x, y, and z directions was built with grid spacing of 0.375 Å. The default settings were used for all other parameters.

6.4.4 Analysis and visualization of docking simulation results

At the end of the docking, the best poses were analyzed for hydrogen bonding and calculation using Discovery studio 4.1 and python software was used to view the structure. From molecular docking study estimated by molecular docking score.

6.4.5 Procedure of in silico studies

- Softwares and Databases used
- Accerlys discovery studio viewer
- Molinspiration server
- Accelrys accord for excel
- RCSB protein data bank
- Online SMILES translator
- Autodock 4.2 which combines
- Autodock tools
- Python molecule viewer 1.5.6
- Cygwin 64

6.4.5.1 STEP I: Protein structure refinement

3PBL, 1JS3, 3EML, 2ZAZ, 20K, 2V5Z enzyme was downloaded from RCSB Protein Data Bank (PDB) and the enzyme was refined before docking. The steps involved are:

- Open Accelrys discovery studio viewer.
- File→Open→Select the enzyme file downloaded from RCSB PDB.

- Click View option and then click Hierarchy.
- Click water molecules.
- Click water molecule →Select all water molecules◊cut.
- Select ligand, which is unnecessary and cut.
- Save the molecule in a desired location

6.4.5.2 STEP II: Ligand file format conversion

- The ligands which are desired are drawn in ChemSketch software.
- Tools →Click Generate→Cick SMILES notation (Simplified Molecular Input Line Entry System, which is a file format).
- Save the SMILES in a word document.
- Open the online smiles translator –cactus. nci.nih.gov/services/ translate/
- Uploadthe SMILES.
- By choosing the required file format and save the file in a pdb format (e.g.:ligand.pdb).
- Online smiles translator allows the user to convert SMILES format into PDB, MOL, SDF and smile text file format. Thus the selected ligand molecule of canonical smile format was converted to pdb format.
- The protein and ligand files which are prepared by above said procedures are taken for docking.

6.4.5.3 STEP III: Docking with autodock 4.2

- Docking calculation in AutoDock was performed using the refined protein And the desired ligand in pdb format.
- Preparation and running a docking programme

6.4.5.3.1 Preparing the protein

- Open autodock 4.2
- Open file→Click read molecule→Choose the particular refined enzyme file.

The elimination of the water is carried by the following steps.

- Press Select option
- Click Select→click select from string option
- Then write “*HOH*” in the Residue line &“*” in the atom line.

- ❖ Click Add→No new selection and then dismiss.

Addition of hydrogens is done by,

- ❖ Press Edit option
- ❖ Click the Hydrogens
- ❖ Then click Add
- ❖ Choose all Hydrogen, No Bond Order, and 'yes' to renumbering→click Ok.
- ➢ Next click edit option→click add the Kollmann Charges.
- ➢ Then save the enzyme molecule as 1ea1refined.pdb
- ➢ Select Edit→Delete→Delete all molecule

6.4.5.3.2 Preparing the ligand

- ➢ Confirm that all the hydrogens are added in the ligand.
- ➢ Toggle the Auto Dock Tools button.
- ➢ Open theLigand →Click Input and choose the suitable ligand file and finally open.

The torsions are designed by following steps,

- ❖ In the Ligand option select Torsion Tree
- ❖ Select Detect Root option
- ❖ Click Torsion Tree
- ❖ Then select the Choose Torsions option
- ❖ Amide bonds should NOT be active.
- ➢ After that click the Torsion Tree and select Set Number of Torsions
- ➢ Number of rotatable bonds is chosen.
- ➢ Finally Save the Ligand files by selecting the Output option (pdbqt file).
- ➢ Select Edit→Delete→Delete all molecule.

6.4.5.3.3 Conversion of pdb files of protein into pdbqt file

- ❖ Select the Grid option and open the Macromolecule pdb file.
- ❖ Auto Dock adds the Charges and itself merges the Hydrogens.
- ❖ Save the object as pdbqt in desired area.

6.4.5.3.4 AutoGrid Calculation and creating "gpf" file

- ➢ Open the grid and click Macromolecule option and choose the rigid protein then yes to preserve the existing charges.

The Preparation of grid parameter file is carried out by,

- ❖ Open Grid
- ❖ Select the Set Map Types
- ❖ Choose Ligand
- ❖ Accept it.

Setting of grid properties,

- ❖ Open Grid
- ❖ Experimental Section
- ❖ Select the Grid box
- ❖ Set the proper Grid Dimensions(60.60.60)
- ❖ Adjust the Spacing
- ❖ Select the File and click Close Saving Current.
- ➢ Save the grid settings as gpf file in the input option (ligand.gpf).
- ➢ After running the grid file, the output automatically save as ‘glg’ file

6.4.5.3.5 Auto Dock calculation and creating ‘dpf’ file

The rigid molecule specification is carried out by,

- ❖ Select the Docking option
- ❖ Click the Macromolecule
- ❖ Set Rigid File Name.

The ligand specification is carried out by,

- ❖ Click the Docking option
- ❖ Select the ligand
- ❖ And then Accept it.
- ➢ In the next step, click Docking option and select Search Parameters in that click Genetic Algorithm and finally accept it.
- ➢ ClickDocking options→Select Docking Parameters→Choose the Defaults.
- ➢ Click Docking option→Select Output and adds Lamarckian Genetic algorithm (LGA).
- ➢ Save the docked settings as ‘dpf’ file in the input option (ligand.dpf)
- ➢ After running the docked file, the output automatically saves as ‘dlg’ file.

6.4.5.3.6 Programming of ‘Auto Grid’ and ‘Auto Dock’ execution

1. Open Cygwin and typed as follows:

- **cd C:**
- **cd cygwin**
- **cd usr**
- **cd local**
- **cd bin**

Program should list out the pdb, pdbqt, gpf and dpf files of an enzyme and Ligand molecule.

2. Then type as:

- **/autogrid4.exe <space> -p <space>ligand.gpf –l <space>ligand.glg**

If a ligand gets into the spacing of the grid, then the execution of this command will be;

'Successful completion'.

3. Then type as:

- **/autodock4.exe<space> -p<space>ligand.dpf –l<space>ligang.dlg**

If the ligand binds to the amino acids through 10 different conformations, then the execution of this command will be;

'Successful completion'.

6.4.6.4 STEP IV: Viewing docking results

6.4.6.4.1 Reading the docking log file .dlg

- Toggle the AutoDock Tools button
- Click Analyze and Open Dockings.
- In the next step, click Analyze option and Conformations then Load.
- Double click on the conformation for to view it.

6.4.6.4.2 Visualizing docked conformations

- Click Analyze and Dockings then play.
- Load dlg file
- Choose the suitable conformations
- In the next step, click Analyze and Docking then Show Interactions.

6.4.6.4.3 Obtaining snap shots of docked pose

- Open the File and Read the Molecule
- Open Analyze→Click Dockings and Open dlg file
- Open Analyze→Click Macromolecule and Choose pdbqt file.

- Open Analyze→Click Conformations and Load
- Double click the desired conformation
- Click Analyze and Docking then Show Interactions.
- Proteins and ligand interaction will be displayed. Zoom it and increase the contrast by holding right key and ctrl.
- Open File→Save image→cygwin/usr/local/bin as.png

6.5. Acute oral toxicity studies (OECD 423 guidelines) of 1, 2, 4-triazin derivative

OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original Guideline 423 was adopted in March 1996 as the second alternative to the conventional acute toxicity test, described in Test Guideline 401. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Animals should be fasted prior to dosing with the mouse, food but not water should be withheld for 3-4 hours. Following the period of fasting, the animals should be weighed and the test substance administered.

After the substance has been administered, food may be withheld for a further 1-2 hours in mice. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 50 mg/kg body weight. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also cardio vascular system, central nervous systems, autonomic nervous and gastro intestinal tract [66, 67].

Till 14th days observation the animals were out of any side effect, the 1, 2, 4-Triazin derivative synthesize drug was selected as *1/10* of the *dose* that was used in the *treatment group.* The doses for in vivo study are selected as 30 mg/kg. **(Figure No. 10)**

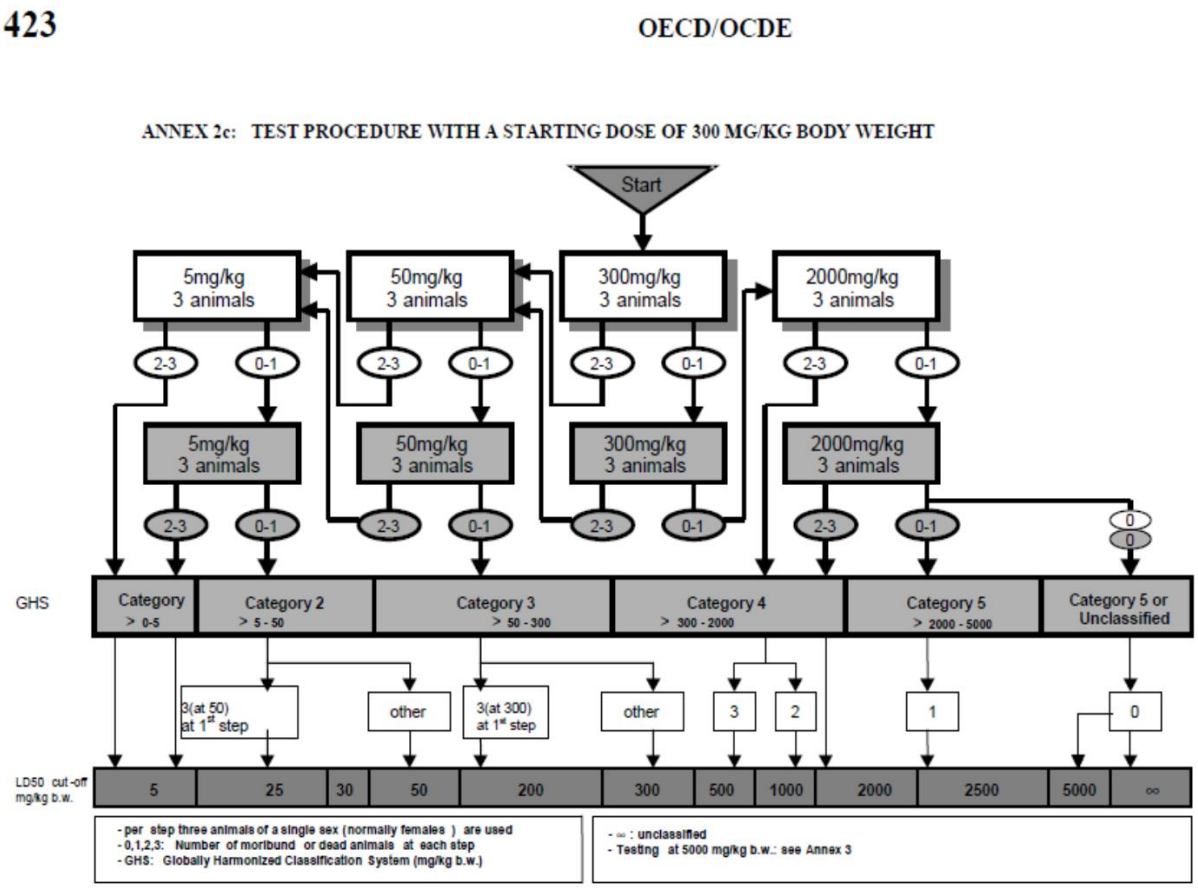


Figure No 10. Test procedure with starting dose of 300mg/kg body weight

6.6 *IN VIVO* ANTI PARKINSON'S STUDIES

6.6.1 SELECTION OF ANIMALS

Healthy adult Male Albino Wister Rats (270-350gm) and Female Swiss Albino Mice (25-30 gm) will be used for the present study. They are housed in poly propylene cages and maintained in an air-conditioned well ventilated animal house with standard lab conditions at temperature (25±2ºC), humidity (50±15%) under 12

hour light/dark cycle and were obtained from central animal house of Swamy Vivekanandha College of Pharmacy. They are fed with standard pellet diet and water ad libitium. All animals used in this study were handled with humane care incompliance with the Indian National Science Academy Guidelines for the use and care of experimental animals in research and all the animal experiments will be performed according to the guidelines prescribed by Committee for the purpose of control and supervision on experiments on animals (CPCSEA).

IAEC Reference No: SVCP/IAEC/PG/1/02/2017

6.6.2 ANIMAL GROUPING

Twenty four Albino Wistar male Rats weighing 250 to 270 gm were randomly divided into four groups of six per each. (4 X 6 = 24 Albino Wistar rats). **(Table No. 3)**

Table No 3. Animal grouping

S.NO	GROUPS	TREATMENT	ANIMALS REQUIRED
1	**Group I** (vehicle group)	Received 1% Gum acacia solution+ Distilled water p.o. for 14 days	**6**
2	**Group II** (Negative control	Received Chlorpromazine 3mg/kg, i.p. (dissolved with 1% gum acacia in distilled	**6**

	group)	water suspension) daily for a period of 14 days	
3	**Group III** (Standard group)	Received Chlorpromazine (3mg/kg/day),i.p. + Diclofenac (10mg/kg/day) p. o. for 14 days	**6**
4	**GroupIV (**Treatment group)	Received Chlorpromazine (3mg/kg/day), i.p. + 1,2,4-Triazin derivative of Diclofenac at dose of 30mg/kg, p. o, respectively for 14 days	**6**
TOTAL NUMBER OF ANIMALS			**24**

6.6.3 INDUCTION OF CHLORPROMAZINE INDUCED PARKINSON'S

Parkinson's disease was induced by intra peritoneal injection of chlorpromazine 3mg/kg, i. p (dissolved with 1% gum acacia in distilled water suspension) daily for a period of 14 days. Chlorpromazine was administered to all treated groups 30 min before the administration of test drug [68].

6.6.4 PHYSICAL EVALUATION:

6.6.4.1 Body weight

Body weight of each rats in all groups were measured weekly till end of the treatment using a weighing balance and the changes were recorded.

6.6.4.2 Feed intake

Daily feed consumption was measured in individual treatment group by using standard weighing balance.

6.6.5 BEHAVIOURAL ASSESSMENT

All animals were tested on behavioural activity of rat tests in familiar environment was monitored on 0^{th} , 7^{th} and 14^{st} day after 30 minutes of drug treatment.

a) Evaluation of exploratory and locomotors activity by using Actophotometer Apparatus

b) Evaluation of muscle coordination behaviour by using Rota Rod
c) Evaluation of cognitive performance by using Morris Water Maze Apparatus
d) Evaluation of catalepsy

6.6.5.1 Evaluation of exploratory and locomotor activity by using Actophotometer Apparatus

To study the locomotor activity of animal behavior was monitored and calculated by using an Actophotometer (activity cage), described by Dews P.B. (1953). In this photoelectric cells joined in circuit with a counter. When a beam of light falls on the photocell is cut off by the rat, a count is recorded. On the study 0^{th} day, 7^{th} day and 14^{st} days the rats from each group were placed individually in the activity cage for 10 mins and the scores of each rat was recorded [69].

6.6.5.2 Evaluation of muscle coordination behaviour by using Rota Rod

Dunham and Miya (1957) suggested that neurological depression in rats could be evaluated by testing their ability to remain on a Rota-rod. Rota-rod apparatus is a four panel techno device with timer. Animals (4 at a time) were placed on rod rotating at 20-25 rpm speed. Only the rat, which demonstrated their ability to remain on the revolving rod (20-25 rpm) for 5 min after training sessions during pretest screening, was selected for studies. Decrease in fall off time is suggestive of CNS depression. On the study 0^{th} day, 7^{th} day and 14^{st} day the rats from each group were fall off time was recorded in all the groups 30 min after drug administration [70, 71].

6.6.5.3 Evaluation of cognitive performance by using Morris Water Maze Apparatus

Cognitive abilities were measured by using Morris Water Maze. The chamber was a black circular swimming pool of nontoxic materials (160 cm diameter, 80cm high and 40 cm deep) and filled with water. Visual cues were placed around the chamber. This chamber was divided into four equal quadrants. A square hidden black platform (10 cm diameter) was submerged beneath (1.5 cm) the water surface in the middle of the target quadrant in the pool. The water is made opaque using titanium dioxide suspension and is kept at about $23^0C \pm 2^0c$ during experiment. Each trial is started from one of four assigned polar positions with a different sequence

each day. The latency to find the platform is measured as the time of placement of the rat in the water to the time it finds the platform. On the study 0th day, 7th day and 14st day the rats from each group were fall off time was recorded in all the groups 30 min after drug administration [72, 73].

6.6.5.4 Evaluation of catalepsy

On the 7th day and 14th day of the experiment the scores were recorded in three stages and the scores for each stage was assigned.

- Rat moves normally when placed on the table, score of 0 was given
- In first stage the rat was pushed on the back, if failed to move a score of 0.5 was given.
- For second stage front paws of each rat was placed alternately on 3 cm high block. If the rat failed to maintain the forced posture in 10 seconds, a score of 0.5 for each paw with a total of 1 for this stage was given.
- For third stage the front paws of each rat was placed alternately on 9 cm high block. If the rat failed to maintain the forced posture in 10 seconds, a score of 1 for each paw with a total of 2 was added to the scores of first and second stage.
- Thus for the single rat, the maximum possible score was 3.5 and that indicate the total catalepsy [74].

6.6.6 BIOCHEMICAL ESTIMATION

On 14th day of the study, the animals were anesthetized with diethyl ether. The blood was drawn through retro orbital plexus, the serum was obtained after centrifugation of total blood without anticoagulants, at 3000 rpm for 10 min the serum was utilization forthe analysis of serum glutamate pyruvate (SGPT), glutamate oxaloacetate transaminase (SGOT), Alkaline phosphates, Total bilirubin, Urea, Creatinine by standard laboratory techniques [75, 76, 77, 78].

6.6.7 Estimation of antioxidant enzyme levels in rat brain.

On 14th day after behavioural quantification all animals were sacrificed by cervical dislocation. The brains were removed, forebrain was dissected out. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4) and it was used for further estimation of antioxidant level.

a) Estimation of superoxide dismutase (SOD)
b) Estimation of reduced glutathione (GSH)
c) Estimation of Nitrite
d) Estimation of Protein
e) Estimation of Lipid Peroxidation Products
f) Estimation of catalase
g) Estimation of brain glutamate level

6.6.7.1 ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)

To 1 ml of the sample, 0.25 ml of absolute ethanol and 0.15 ml of chloroform were added. After 15 min of shaking in a mechanical shaker, the suspension was centrifuged and the supernatant obtained constituted the enzyme extract. The reaction mixture for auto-oxidation consisted of 2 ml of buffer, 0.5 ml of 2 mM pyrogallol and 1.5 ml of water. Initially the rate of auto-oxidation of pyrogallol was noted at an interval of 1 min for 3 min. the assay mixture for the enzyme contained 2ml of 0.1 M Tris - HCl buffer, 0.5 ml of pyrogallol, aliquots of the enzyme preparation and water made up to 4 ml. The rate of inhibition of pyrogallol auto-oxidation after the addition of the enzyme was noted. The superoxide dismutase activity was measured by the inhibition of pyrogallol auto-oxidation at 420 nm for 10 min. One unit of superoxide dismutase is the amount of enzyme required to bring about 50% inhibition of auto-oxidation by pyrogallol. The enzyme activity was expressed in terms of units/min/mg protein [79].

6.6.7.2 ESTIMATION OF REDUCED GLUTATHIONE (GSH)

1ml of tissue homogenate was precipitated with 1 ml of 10% TCA. The precipitate was removed by centrifugation. To an aliquot of the supernatant was added 4 ml of phosphate solution and 0.5 ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) reagent were added and absorbance was taken at 412 nm. The concentration of GSH in the supernatant was determined from the standard curve using standard reduced glutathione and expressed in nM/mg of protein [80].

6.6.7.3 ESTIMATION OF NITRITE

The production of nitric oxide (NO) in the brain may occur due to oxidative stress and it can be determined by estimation of nitrite level. The nitrite level was

determined spectrophotometrically with Griess reagent (0.1% N-1-naphthyl ethylene amine dihydrochloride, 1% sulphanilamide, and 2.5% phosphoric acid). Brain homogenate and Griess reagent were mixed equally and this mixture was incubated for 10 min and the absorbance was measured at 546nm.The standard curve of sodium nitrite was prepared and the concentration of nitrite in the supernatant was determined from standard curve [81].

6.6.7.4 ESTIMATION OF PROTEIN

The protein content of brain tissue was estimated by method described by Lowry et al. Standard curve was determined using bovine serum albumin and values are expressed in mg/ml [82].

6.6.7.5 ESTIMATION OF LIPID PEROXIDATION PRODUCTS

Lipid peroxidation was estimated spectrophotometrically in brain tissue by quantifying TBARS. In brief, for the estimation of TBARS the supernatant of the tissue homogenate was treated with Thio barbituric acid - Tri chloro acetic acid, (TBA–TCA) reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15 minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant taken for measurement. The developed colo was read at 532 nm using a UV spectrophotometer against a reagent blank. The concentration of TBARS in the supernatant was determined from the standard curve using 1, 1, 3, 3-Tetra Methoxy Propane (TMP) and expressed in nM/mg of protein [83].

6.6.7.6 ESTIMATION OF CATALASE

Homogenized the tissue with M/15 phosphate buffer at 1 to 4°C and centrifuged. Stirred the sediment with cold phosphate buffer and allowed to stand in the cold condition with occasional shaking. Repeat the extraction once or twice, supernatants are combined and used for assay. 3 ml of H_2O_2 phosphate buffer was taken in one cuvette added 0.01 – 0.04 ml sample and read against a control cuvette containing enzyme solution without H_2O_2 phosphate buffer at 240nm.Δt was noted for a decrease in the optical density from 0.450 to 0.400. This value was used for the calculations [84].

6.6.7.7 ESTIMATION OF BRAIN GLUTAMATE LEVEL

Weighed quantity of brain portion was homogenized with 2 parts by weight of perchloric acid and centrifuge for 10 minutes at 3000 rpm. Adjust 3.0ml supernatant fluid to pH 9 with 1.0ml phosphate solution. Allow to stand 10 min. in an ice bath and then filter through a small, fluted filter paper. Allow to warm to room temperature, dilute 1:10 and take 1.0 ml for the assay. Absorbance was measured at 340nm. Similarly a blank reading at 340nm was measured. The level of glutamate was expressed as µmol/g tissue [85].

6.6.8 ESTIMATION OF BRAIN TISSUE EXTRACT NEUROTRANSMITTERS

Examined antioxidant with a help of forebrain, remaining left out brain tissue used to analysis neurotransmitters. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The tissue homogenized was taken in 3 ml HCl-Butanol in a cool environment. The sample was then centrifuged for 10 min at 2000 rpm. 0.8 ml of supernatant phase was removed and added to an eppendorf reagent tube containing 2 ml of heptane and 0.25 ml 0.1 M HCl. After 10 min, shake the tube and centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase was used for dopamine assay.

6.6.8.1 Estimation of brain Ach levels

To 0.4 ml aliquot of the homogenate is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance is measured at 412nm in a photoelectric colorimeter (H2 grade). When absorbance reaches a stable value, it is recorded as the basal reading 5.20 ml of substrate i.e., acetylthiocholine is added and change in absorbance is recorded for a period of 10 min at intervals of 2mins. Change in the absorbance per minute is thus determined [86].

6.6.8.2 Estimation of dopamine assay

To 0.02ml of the HCl phase, 0.005 ml 0.4 ml HCl and 0.01ml EDTA/ Sodium Acetate buffer (pH 6.9) were added, followed by 0.01 ml iodine solution for oxidation. The reaction was stopped after 2 min by the addition of 0.1ml sodium thiosulphate in 5 M Sodium hydroxide. 10 M Acetic acid was added 1.5 min later. The solution was then heated to 100^{o}C for 6 min. When the samples again reach room temperature, excitation and emission spectra were read (330 to 375 nm) in a spectrofluorimeter.

Compared the tissue values (fluorescence of tissue extract minus fluorescence of tissue blank) with an internal reagent standard (fluorescence of internal reagent standard minus fluorescence of internal reagent blank). Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium thiosulphate before iodine). Internal reagent standards were obtained by adding 0.005 ml bidistilled water and 0.1 ml HCl Butanol to 20 ng of dopamine standard [87].

6.6.9 DETERMINATION OF ULCEROGENECITY

At the end of the study stomach of the entire animals were cut opened and the ulcerogenecity was asseses by ulcer score as follows.

0-Normal Mucosa,

0.5-Red coloration,

1.0-Spot ulcers,

1.5-Hemorrhagic streaks,

2.0-Ulcers >3 but <5,

2.5- Ulcer >5.

6.6.10 HISTOPATHOLOGICAL EVALUATION

All the animals were sacrificed ay the end of the experiments. substantia nigra region of brain was removed of all the animals and post fixed in formal saline (24 hrs) washing was done in tape water then serial dilution (methyl, ethyl and absolute) were used to dehydration. Specimens were cleared in xylene and embedded in paraffin at 56º c in hot air oven for 24 hrs. Blocks were prepared by using paraffin wax at 4µm thick in microtone. The obtained tissue section were deparaffinized and stained by hematoxylin and eosin (H&E) stain for histopathological examination through the light microscope [88].

6.7 STATISTICAL ANALYSIS

The statistical analyses were carried out using SPSS version 20.0 for Windows. Behavioral comparisons of pre- and post treatment four groups of data were analyzed using three way analysis of variance (ANOVA) followed by scheffe test for comparisons between various treated groups. The results were presented as

means ± SEM, n=6. Values with $p<0.05$ were considered to be statistically significant [89].

CHAPTER-7

RESULT

CHAPTER 7

RESULT

7.1 Synthetic Scheme of Designed Compounds

2-[(2, 6-dichloroanilino) phenyl] acetic acid (a) was prepared from Diclofenac sodium by hydrolysis in the presence of conc.H2SO4 and ethanol. Ethyl - [2-(2, 6-dichloroanilino) phenyl] acetate (b) was prepared from Compound a by etherification in the presence of conc. H2SO4 and ethanol. [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide (c) was prepared from Compound b by Treatment with Hydrazine Hydrate in Absolute Ethanol. The reaction of Compound c was refluxed with 2-Chloroacetamide and Dimethylformamide to yield 3-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 6-dihydro-1, 2, 4-triazin-5(2H)-one. (d) **(Figure No.: 11)**

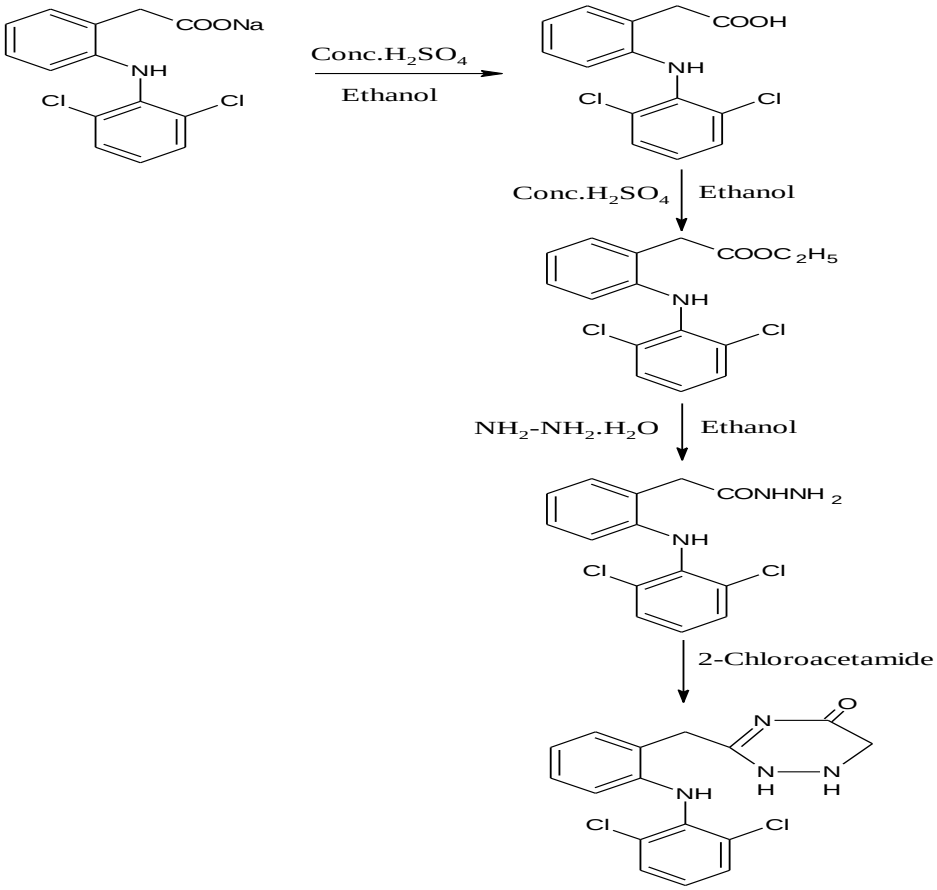


Figure No.: 11 Synthesis of 3-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 6-dihydro -1, 2, 4-triazin-5(2H)-one.

7.2 FT-IR Analysis of Diclofenac Analogues

Compound a: The IR interpretation of 2-[(2, 6-dichloroanilino) phenyl] acetic acid. IR (KBr), v, cm-1: 3100-3000 (CH), 1795.60 (C=O), 3469.70 (O-H), 1419.51 (C-O-H), 3095.54 (N-H), 1367.44 (C-N), 2509.22 (CH_2). **(Figure No.: 12)**

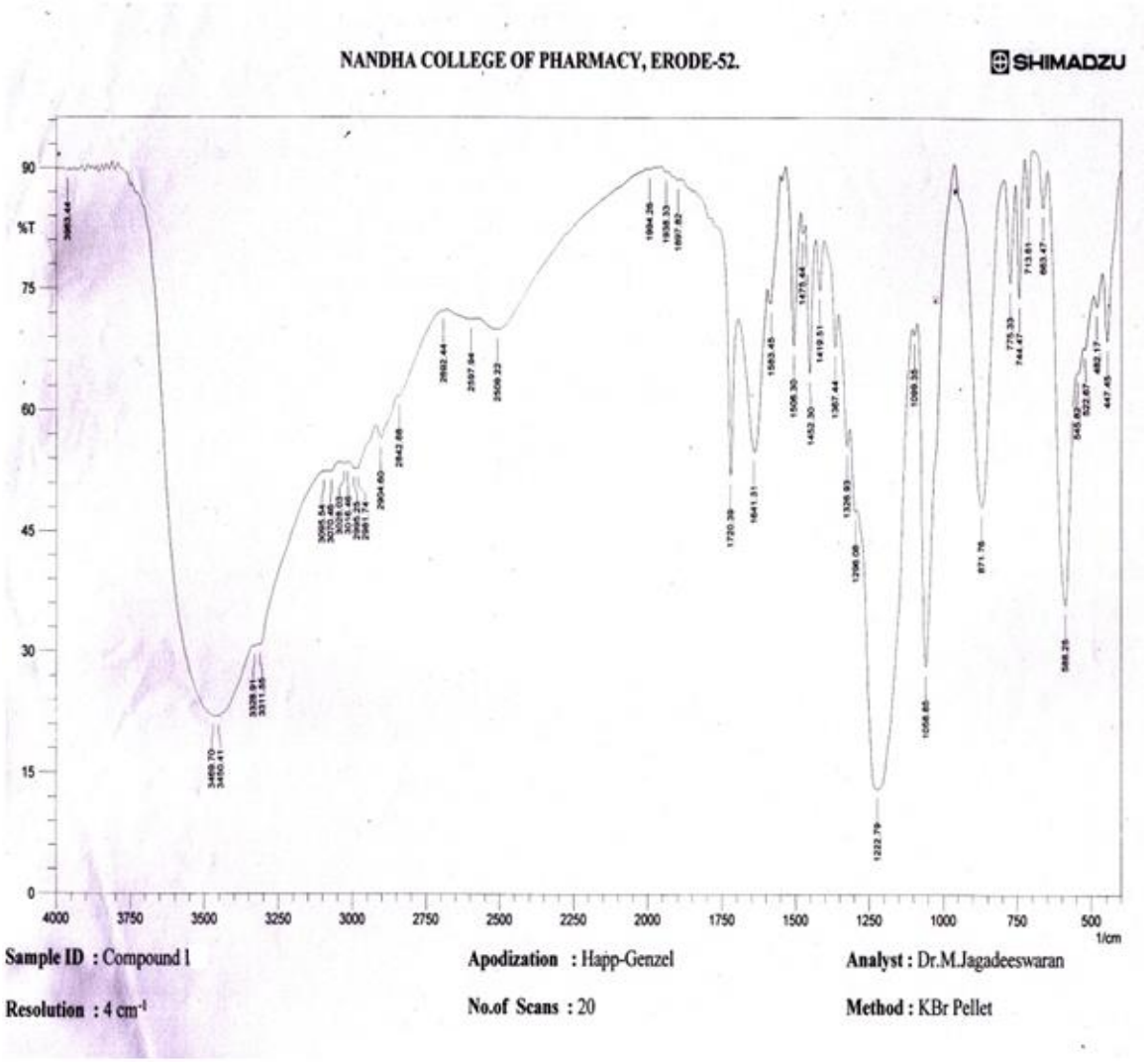


Figure No.: 12 IR spectrum of compound-a (2-[(2, 6-dichloroanilino) phenyl] acetic acid)

Compound b: The IR interpretation of ethyl - [2-(2, 6-dichloroanilino) phenyl] acetate. IR (KBr), v, cm-1: 3307.69 (CH), 1720.39 (C=O), 1238.21 (C-O-H), 3417.63 (N-H), 1326.93 (C-N), 2981.74 (CH_2), 482.17 (C-Cl). (**Figure No.: 13)**

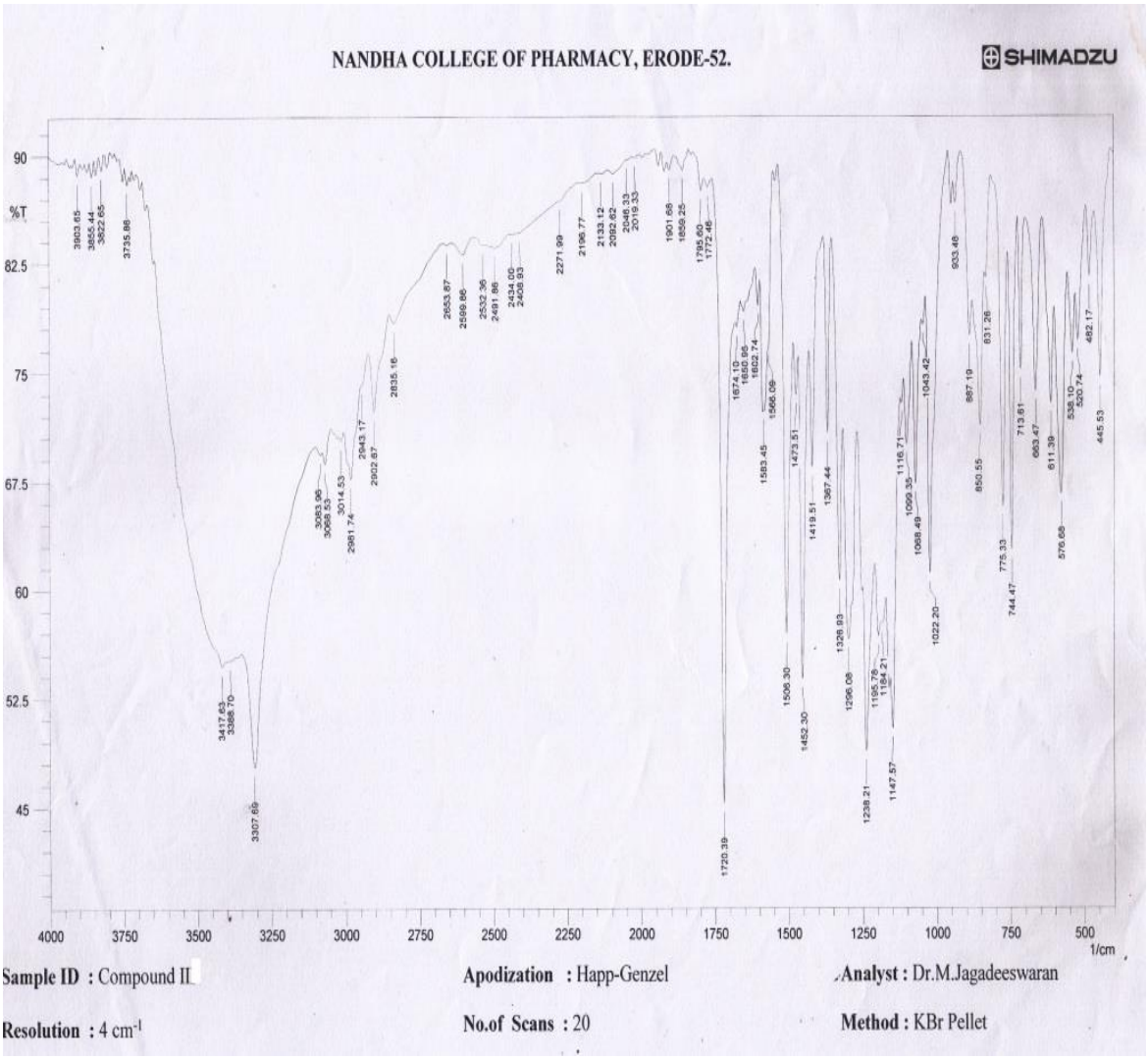


Figure No.: 13 IR spectrum of compound-b ethyl - [2-(2, 6-dichloroanilino) phenyl] acetate

Compound c: The IR interpretation of [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide. IR (KBr), v, cm-1: 3056.96 (CH), 1488.94 (C=Cl), 1731.96 (C=O), 3450.41 (NH), 1240 (C-N), 3417.63 (N-H), 1326.93 (C-N), 1488.94 (CH_2), 455.03 (C-Cl). (**Figure No.: 14)**

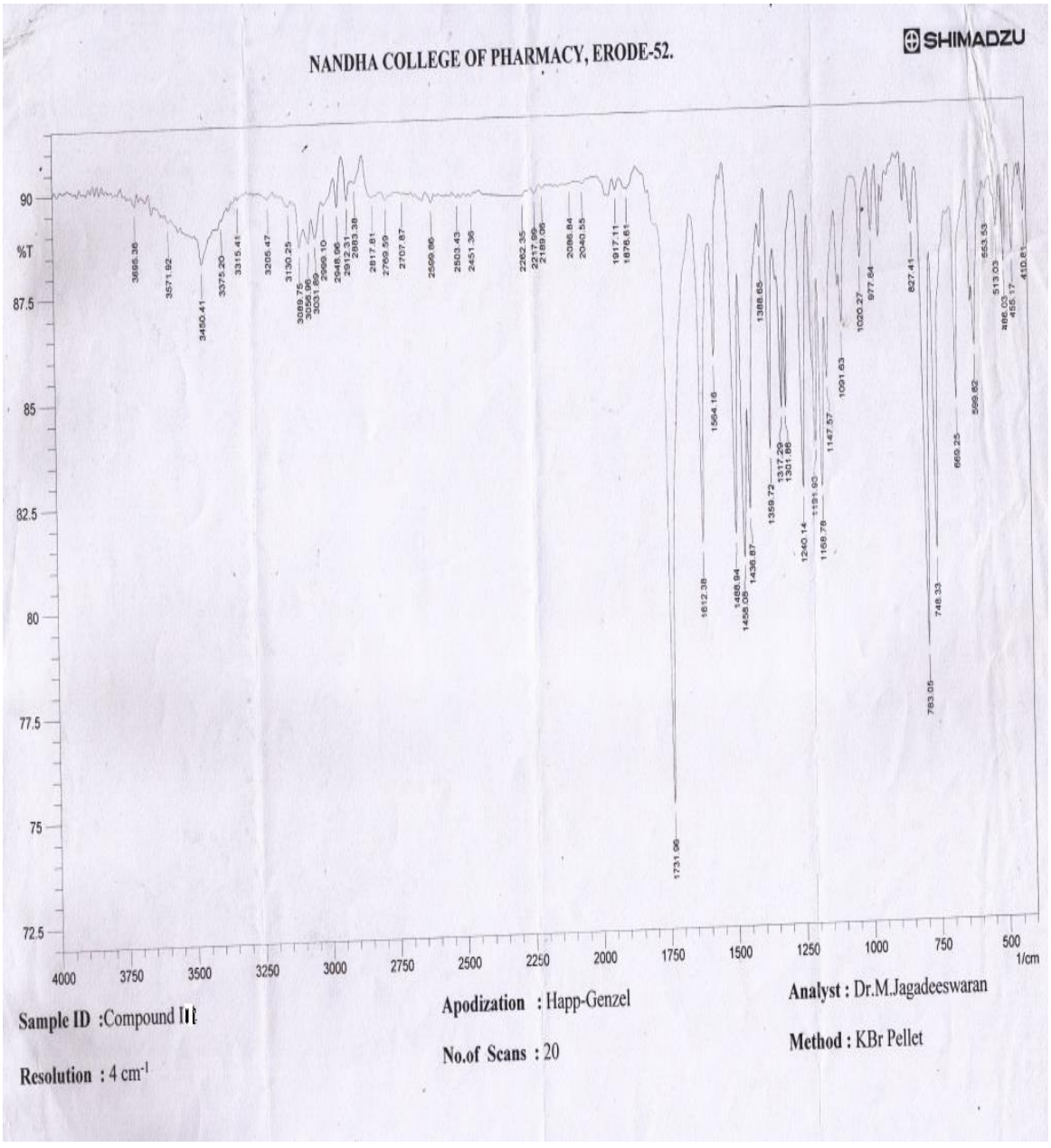


Figure No.: 14 IR spectrum of compound-c [2-(2, 6-dichloroanilino) phenyl] acetic acid hydrazide

Compound d: The IR interpretation of IR (KBr), v, cm-1: 3056.96 (CH), 1488.94 (C=Cl), 1731.96 (C=O), 3450.41 (NH), 1240 (C-N), 3417.63 (N-H), 1326.93 (C-N), 1488.94 (CH_2), 455.03 (C-Cl). (**Figure No.: 15)**

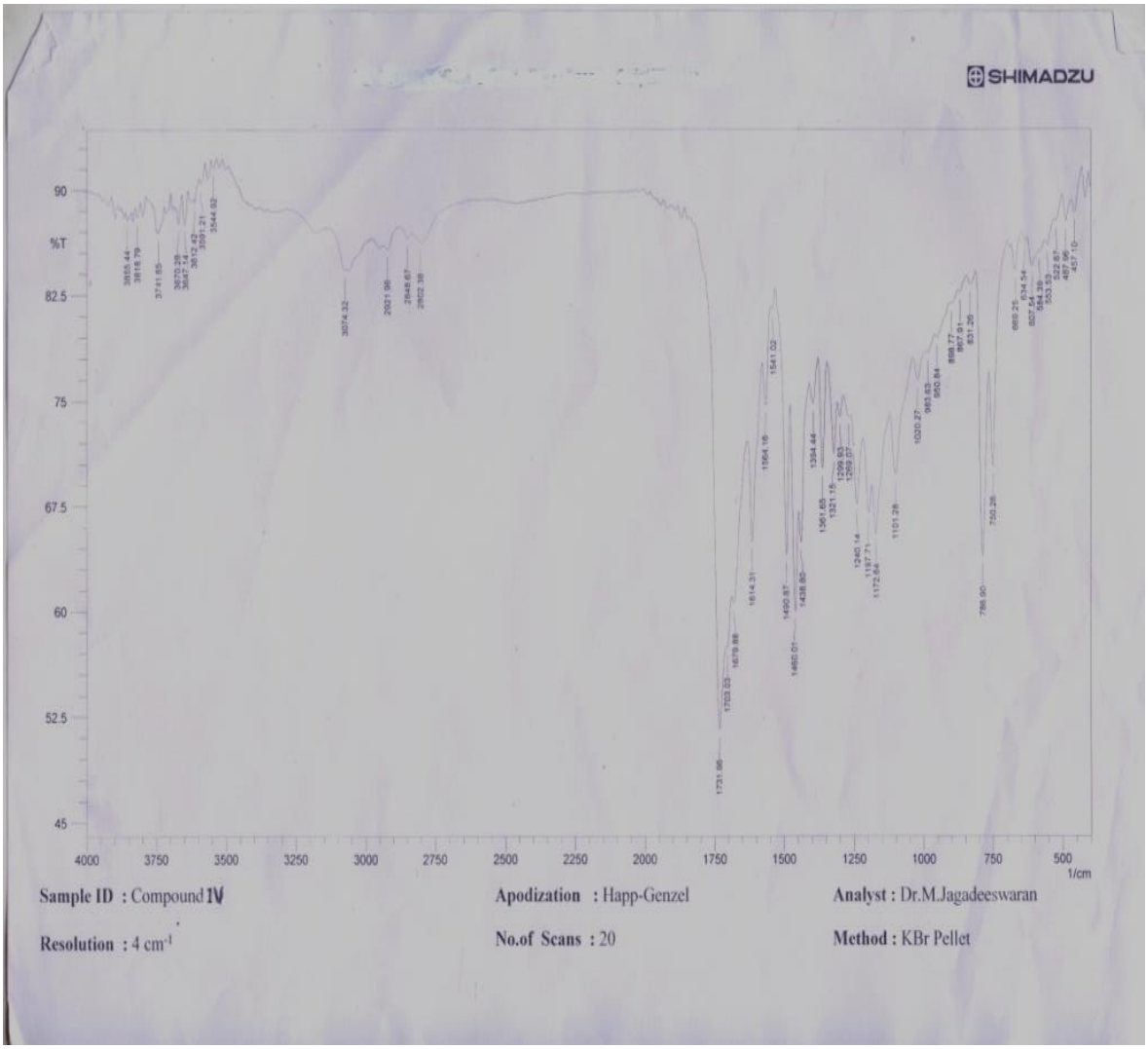


Figure No.: 15 IR spectrum of compound-d 3-{2-[(2, 6-dichlorophenyl) amino] benzyl}-1, 6-dihydro-1, 2, 4-triazin-5(2H)-one.

In this above synthesized Compounds a, b, c and d entire IR spectra region used to identification the functional group. The compounds showed the possible IR (KBr), v, cm-1: CH, C=O, C-O-H, N-H, C-N, CH_2, C-Cl shows the presence of the functional group.

7.3 Molecular Docking studies of 1, 2, 4-Triazin derivative of diclofenac on Molecular Docking

Structure of 1, 2, 4- Triazin derivative of Diclofenac was drawn by using chemoffice 2004 software and docking simulation was carried out against the Parkinson's enzyme targets like Dopamine receptor D3 protein (3PBL), (**Figure No .16)** Dopa decarboxylase-DDC (1JS3), (**Figure No .17)** Adenosine A2 receptor-AA2AR (3EML), (**Figure No .18)** P38 map kinase (2ZAZ), (**Figure No .19)** Monoamino oxidase-B-MAO-B (2V5Z) (**Figure No .20)** enzymes target with the help of Autodock k4 program. The binding scores of designed ligand was 1-10 scores with D_3 protein, DDC, $AA_{2A}R$, MAPK and MAO-B enzymes ranging from -6.35 to -5.64 Kcal/mol, -6.86 to -5.81 Kcal/mol, -6.11 to -5.02, -8.67 to -4.63 Kcal/mol, and -10.25 to -6.76 Kcal/mol respectively. **(Table No. 4)**

Table No.: 4 Docking scores for 1, 2, 4-Triazin derivative of diclofenac against the Parkinson's enzyme targets

Target Name	PDB Code	Docking Score (Kcal/Mol)
Dopamine receptor D3 protein	3PBL	-6.35 to -5.64
Dopa decarboxylase (DDC)	1JS3	-6.86 to -5.81
Adenosine A2 receptor ($AA_{2A}R$)	3EML	-6.11 to -5.02
P38 map kinase (MAPK)	2ZAZ	-8.67 to -4.63
Monoamino oxidase-B (MAO-B)	2V5Z	-10.25 to -6.76

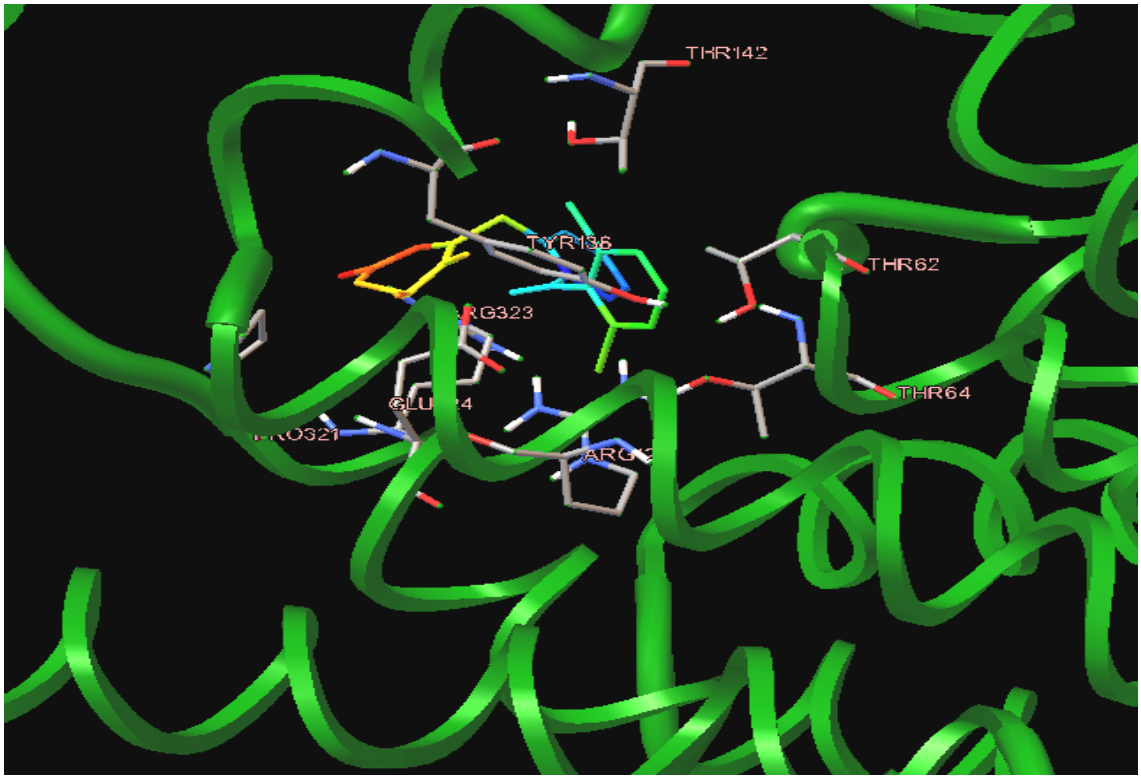


Figure No.: 16 Docking study of 1, 2, 4-Triazin derivative of diclofenac against 3PBL targets

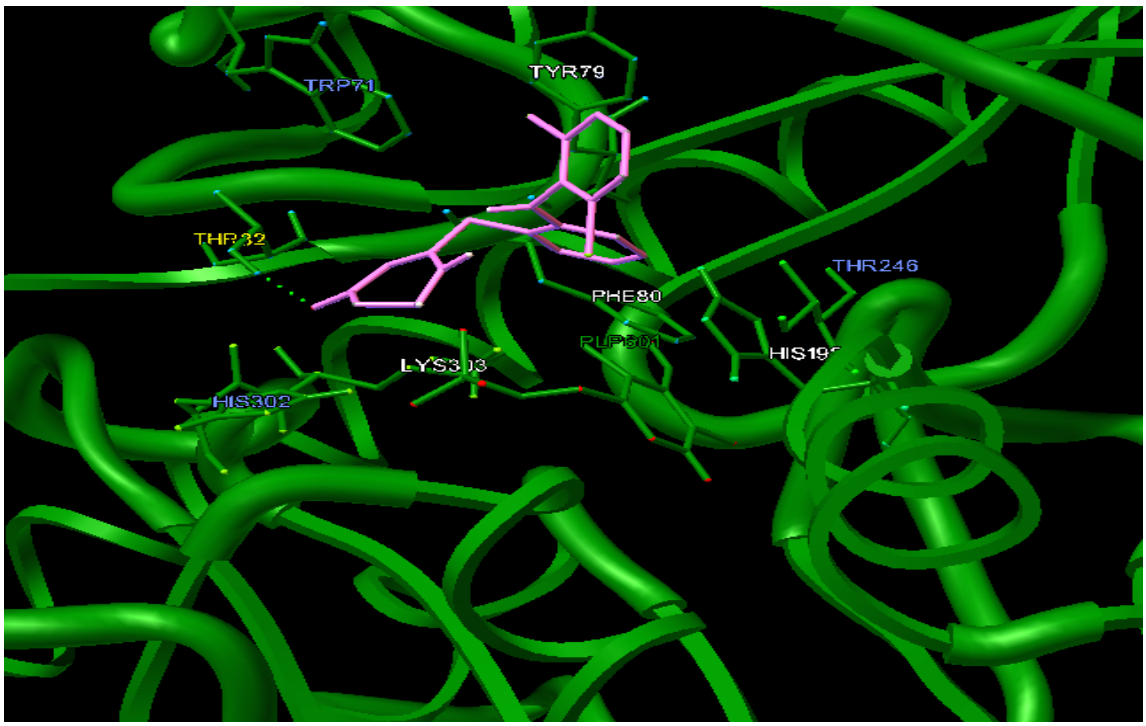


Figure No.: 17 Docking study of 1, 2, 4-Triazin derivative of diclofenac against 1JS3 targets

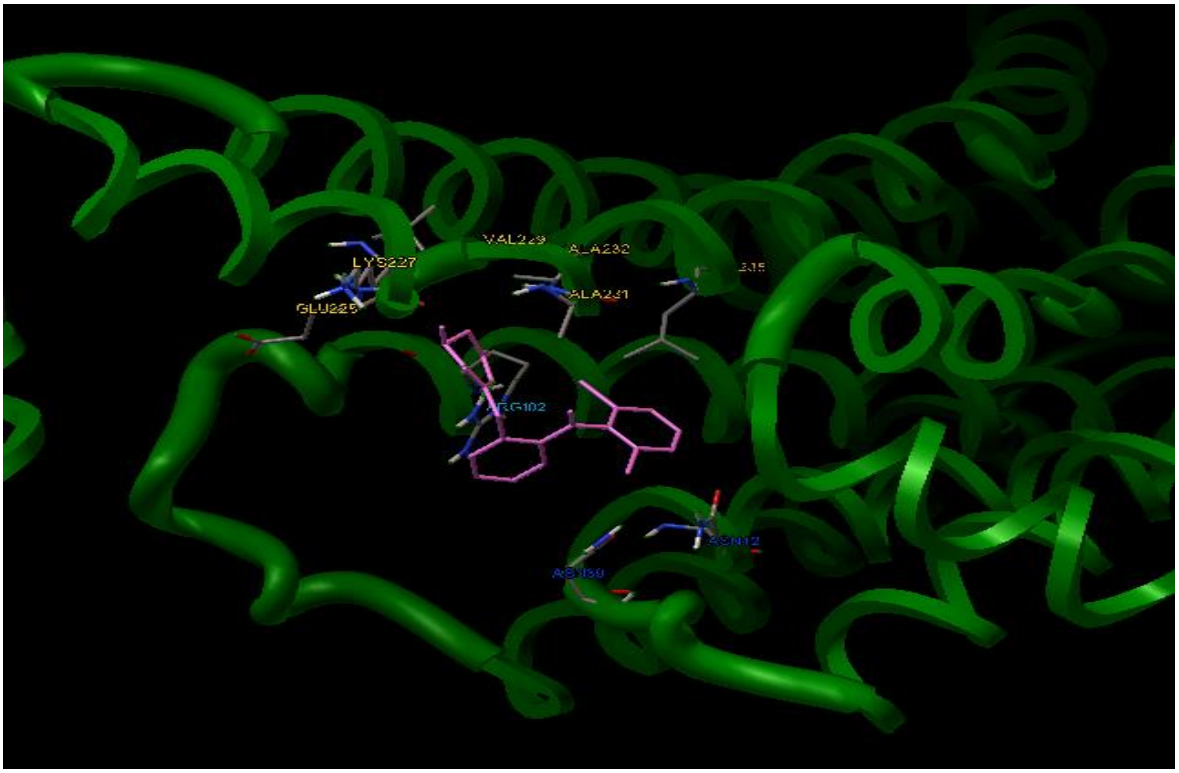


Figure No.: 18 Docking study of 1, 2, 4-Triazin derivative of diclofenac against 3EML targets

Figure No.: 19 Docking study of 1, 2, 4-Triazin derivative of diclofenac against 2ZAZ targets

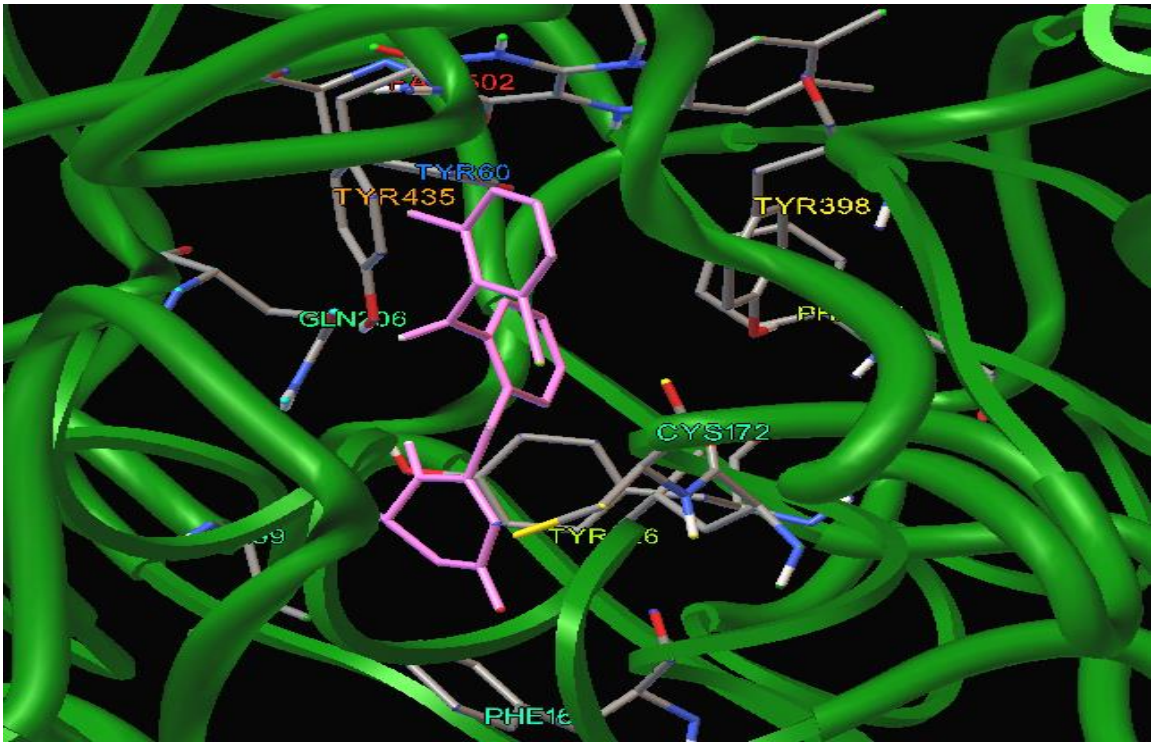


Figure No.: 20 Docking study of 1, 2, 4-Triazin derivative of diclofenac against 2V5Z targets

7.4 Effect of 1, 2, 4-Triazin Derivative on Changes in Acute Oral Toxicity Studies

The 1, 2, 4-Triazin derivative compound not produced any toxic symptoms or mortality up to dose level of 300mg/kg orally in mice and hence, the drugs were considered safe for further pharmacological screening. As per OECD-423 1/10 (30mg/kg) of 1, 2, 4-Triazin derivative was used for future pharmacological screening. No lethal toxic reactions were observed until the end of the 14 days. **(Table No.: 5)**

Table No.: 5. Acute oral toxicity study of 1, 2, 4-Triazin derivative of Diclofenac

S. No	Symptoms	1, 2, 4-Triazin Derivative Compound (30mg/Kg, p. o)
1.	Depth of breathing	--
2.	Abdominal breathing	--
3.	Gasping	--
4.	Tachyphea	--
5.	Bradycardia	--
6.	Tachycardia	--
7.	Convulsion	--
8.	Somnolence	++
9.	Loss of righthing refleux	--
10.	Catalepsy	--
11.	Unusual locomototion	--
12.	Sedation	--
13.	Salivation	--
14.	Droping feces	--
15.	Dieresis hematuria	--

7.5 Effect of the 1, 2, 4-Triazin derivative of diclofenac on Changes in Body Weight

On this study, final body weight of chlorpromazine Group II showed significantly ($P<0.05$) decrease in body weight when compare to control Group I. Group III showed mild significant ($P<0.05$) increase in body weight when compared to the Group II. The treatment Group IV are more significant ($P<0.001$) increases when compared to Group II. **(Table No.: 6)**

7.6 Effect of 1, 2, 4-Triazin derivative of diclofenac on Feed Intake in Chlorpromazine Induced Parkinson's Rat

In this study, Feed intake Group II was significantly decreased when compared to control group (Group I). The Diclofenac group (Group III) and treatment Group shows significantly increase in feed intake when compared to Group II. **(Table No.: 7)**

7.7 Effect of 1, 2, 4-Triazin derivative of diclofenac on Exploratory and Locomotor Activity by Using Actophotometer in Chlorpromazine Induced Parkinson's Rat

Chlorpromazine group showed significantly ($P<0.05$) decrease in locomotor activity when compared to the control group. Diclofenac Group III shows significantly increases when compared to chlorpromazine Group II. In this no significant difference in treatment Group IV on the 7^{th} day

On the 14^{th} day, Diclofenac Group III shows significantly ($P<0.05$) increase when compared to control Group I. Treatment Group IV exhibited ($P<0.001$) significantly increases when compared to chlorpromazine Group II and Diclofenac Group III. **(Table No.: 8)**

7.8 Effect of The 1, 2, 4-Triazin derivative of diclofenac on Muscle Coordination Behaviour by Using Rota Rod in Chlorpromazine Induced Parkinson's Rat

The mean fall- off time of Group I animals from the rota rod was observation of the treatment. On the 7^{th} day Group II showed significantly ($P<0.05$) decrease when compare to the control Group I. treatment group IV shows significantly ($P<0.001$) when compared to the chlorpromazine Group IV.

On the 14th day chlorpromazine Group II showed significantly (P<0.001) decreased when compare to the control Group I. Diclofenac group III showed significantly (P<0.001) increased when compare to the chlorpromazine Group II. Treatment Group IV showed significantly (P<0.001) increases when compare to the chlorpromazine Group II. **(Table No.: 9)**

7.9 Effect of The 1, 2, 4-Triazin derivative of diclofenac on Cognitive Performance by Using Morris Water Maze in Chlorpromazine Induced Parkinson's Rat

Antipsychotic related effects on swim speeds on the 7th day chlorpromazine Group II showed significantly (P<0.05) increased when compared to control Group I. Group III showed significant (P<0.05) decreased when compared to Chlorpromazine Group II. Treatment Group IV showed significantly (P<0.001) increase when compared to the chlorpromazine Group II.

On the 14th day chlorpromazine Group II showed significantly (P<0.01) increased when compared to the control Group I. Diclofenac Group III showed significantly (P<0.001) decreased when compared to the chlorpromazine Group II. Treatment Group IV showed significantly (P<0.001) decreased when compared to chlorpromazine Group II. **(Table No.: 10)**

7.10 Effect of The 1, 2, 4-Triazin derivative of diclofenac on Catalepsy in Chlorpromazine Induced Parkinson's Rat

On 7th day chlorpromazine Group II showed significantly (P<0.001) increased when compared to control Group I. Diclofenac Group III shows significantly (P<0.001) decreased when compared to chlorpromazine Group II. Treatment Group IV showed significantly (P<0.001) decreased when compared to chlorpromazine Group II.

On 14th day chlorpromazine Group II showed significantly (P<0.001) increased when compared to control Group I. Diclofenac Group III shows significantly (P<0.001) decreased when compared to chlorpromazine Group II. Treatment Group IV showed significantly (P<0.001) decreased when compared to chlorpromazine Group II. **(Table No.: 11)**

7.11 Effect of 1, 2, 4-Triazine derivative of diclofenac on biochemical parameter In Chlorpromazine Induced Parkinson's Rat

In this study, serum glutamate pyruvate (SGPT), glutamate oxaloacetate transaminase (SGOT), Alkaline phosphates, Total bilirubin, was of chlorpromazine Group II showed significantly (P<0.001) increase in SGPT, SGOT, ALP, Total bilirubin level when compared to control Group I. In the 1, 2, 4-Triazin treatment group shows significantly low level when compared to the Diclofenac Group. **(Table 12)**

7.12 Effect of 1, 2, 4-Triazine derivative of diclofenac on superoxide dismutase (SOD) in Chlorpromazine Induced Parkinson's Rat

In this study, SOD of chlorpromazine Group II showed significantly (P<0.001) decreases in SOD level when compared to control Group I. Diclofenac Group III showed significantly (P<0.01) increases in SOD level when compared to chlorpromazine Group II. The treatment Group IV showed significantly (P<0.01) increases in SOD level when compared to chlorpromazine Group II. **(Table 13)**

7.13 Effect of 1, 2, 4-Triazine derivative of diclofenac on reduced glutathione (GSH) in Chlorpromazine Induced Parkinson's Rat

In this study, GSH of chlorpromazine Group II showed significantly (P<0.001) decreases in GSH level when compared to control Group I. The treatment Group IV showed significantly (P<0.001) increases in GSH level when compared to control Group I. Diclofenac Group III showed significantly (P<0.001) increase in SOD level when compared to chlorpromazine Group II. Treatment Group IV showed significantly (P<0.05) increase in SOD level when compared to Diclofenac Group III. **(Table 13)**

7.14 Effect Of 1, 2, 4-Triazine derivative of diclofenac on nitrite in Chlorpromazine Induced Parkinson's Rat

In this study, nitrite of chlorpromazine Group II showed significantly (P<0.01) increase in nitrite level when compared to control Group I. Diclofenac Group III showed significantly (P<0.05) decrease in nitrite level when compared to chlorpromazine Group II. Treatment Group IV showed significantly (P<0.001) decrease in nitrite level when compared to chlorpromazine Group II. **(Table 13)**

7.15 Effect of 1, 2, 4-Triazine derivative of diclofenac on protein in Chlorpromazine Induced Parkinson's Rat

In this study, protein of chlorpromazine Group II showed significantly ($P<0.001$) decrease in protein level when compared to control Group I. Diclofenac Group III showed significantly ($P<0.01$) increase in protein level when compared to chlorpromazine Group II. Treatment Group IV showed significantly ($P<0.001$) increase in protein level when compared to chlorpromazine Group II. **(Table 13)**

7.16 Effect of 1, 2, 4-Triazine derivative of diclofenac on lipid peroxidation products in Chlorpromazine Induced Parkinson's Rat

In this study, lipid peroxidation of Diclofenac Group III showed significantly ($P<0.01$) increase in lipid peroxidation level when compared to chlorpromazine Group II. Treatment Group IV showed significantly ($P<0.05$) decrease in lipid peroxidation level when compared to chlorpromazine Group II. And Group I and Group II showed no significant difference in lipid peroxidation level when compared to control Group I. **(Table 13)**

7.17 Effect of 1, 2, 4-Triazine derivative of diclofenac on catalase in Chlorpromazine Induced Parkinson's Rat

In this study, catalase of Chlorpromazine Group II showed significantly ($P<0.001$) decrease in catalase level when compared to control Group I. Treatment Group IV showed significantly ($P<0.05$) increase in catalase level when compared to control Group I. And then Diclofenac Group III and chlorpromazine Group II showed significant ($P<0.001$) decreases in catalase level when compared to Diclofenac Group II. **(Table 13)**

7.18 Effect of 1, 2, 4-Triazine derivative of diclofenac on brain glutamate level in Chlorpromazine Induced Parkinson's Rat

In this study, brain glutamate level of Chlorpromazine Group II showed significantly ($P<0.01$) increases when compared to control Group I. Diclofenac group III showed significantly ($P<0.001$) decrease when compared to control Group I. Treatment Group IV showed significantly ($P<0.001$) decrease when compared to control Group I. Diclofenac Group III showed significantly ($P<0.001$) increases when compared to Chlorpromazine Group II. Treatment Group IV showed significantly ($P<0.01$) increase when compared chlorpromazine Group II. **(Table 13)**

7.19 Effect of 1, 2, 4-Triazin derivative of diclofenac on Brain Ach Levels in Chlorpromazine Induced Parkinson's Rat

In this brain acetylcholine chlorpromazine group showed significantly ($P<0.01$) increase when compared to control group. Treatment group showed significantly ($P<0.05$) decrease when compare to Chlorpromazine group. Diclofenac group showed no significant difference when compared to control group. **(Table 14)**

7.20 Effect Of 1, 2, 4-Triazin derivative of diclofenac on Dopamine Assay in Chlorpromazine Induced Parkinson's Rat

The dopamine level in chlorpromazine animals were reduced significantly ($P<0.001$) decrease when compared to control Group I. Treatment Group IV showed significantly ($P<0.001$) increase when compared to Diclofenac Group II. And then Treatment Group IV showed significantly ($P<0.01$) increase when compared to chlorpromazine Group III. **(Table 14)**

7.21 Effect of 1, 2, 4-Triazin derivative of diclofenac on Determination of Ulcerogenecity in Chlorpromazine Induced Parkinson's Rat

In ulcerogenecity chlorpromazine induced Group II shows ulcer score denoted as 0.5. In this Diclofenac Group III shows ulcer score was increased were denoted as 2.0. Treatment Group IV and control Group I shows ulcer score was denoted as normal 0. **(Figure No. 21)**

GROUP I

GROUP II

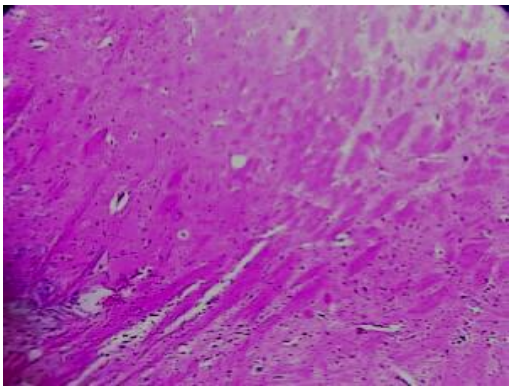
GROUP III

GROUP IV

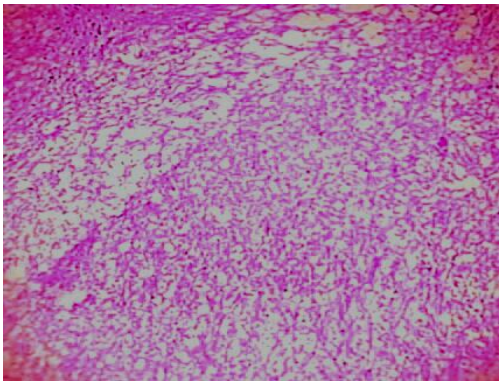
Figure No.: 21 Effect of 1, 2, 4-triazin derivative of diclofenac on determination of ulcerogenecity in chlorpromazine induced parkinson's rat

7.22 Histopathological evaluation

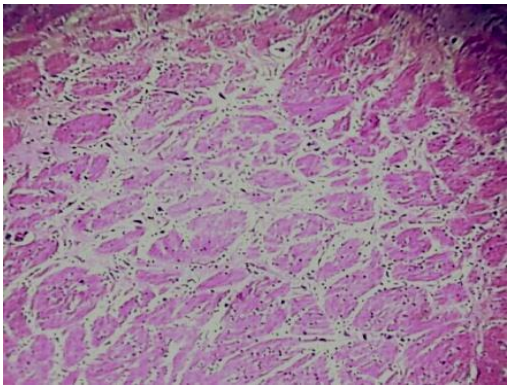
Histopathology of our samples showed that chlorpromazine caused degeneration in the mid brain region of the rats as neurons were under oxidative stress when compared with normal rats as can be seen from Presence of hyper chromatic nuclei with eosinophilic vacuolated cytoplasm in edematous. However sections examined from 1, 2, 4-Triazin derivative treated group shows exhibits intact architecture with occasional hyperchromatic nuclei and mild vacuolization suggestive of mild degenerative changes in treated animals. **(Figure No. 22)**



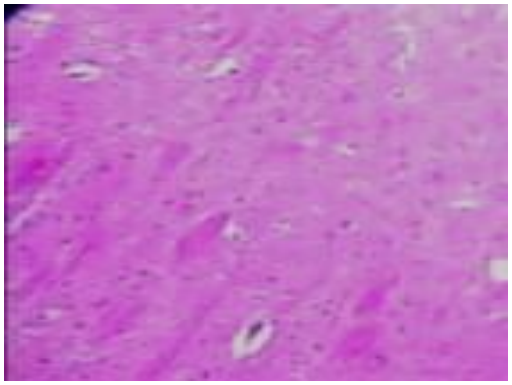
CONTROL GROUP



DICLOFENAC GROUP



CHLORPROMAZINE GROUP



1, 2, 4- TRIAZIN TEATMENT GROUP

Figure No.: 22 Histopathological evaluations

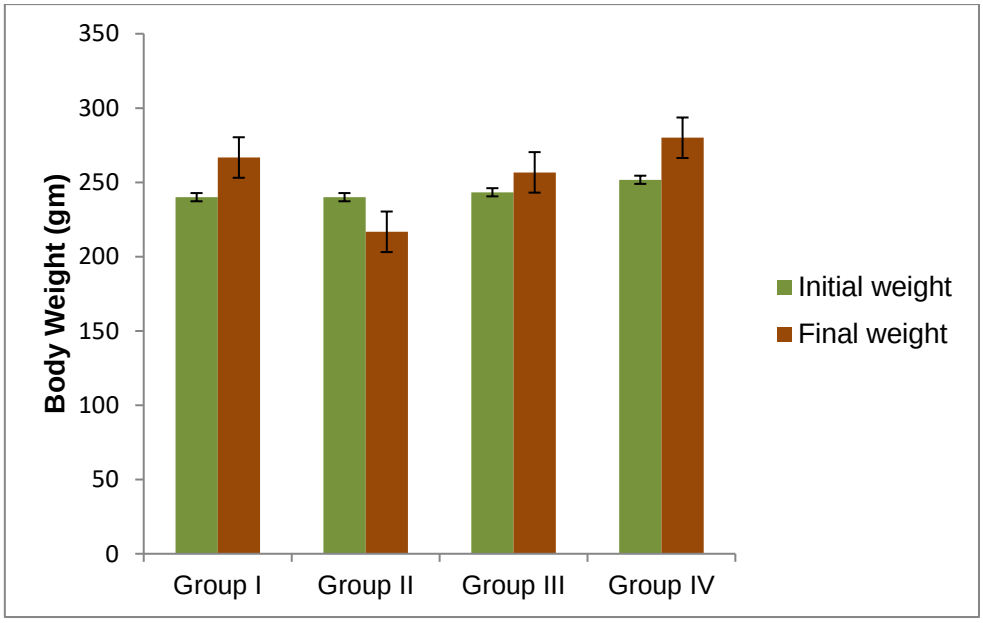
Table No.: 6 Effect of 1, 2, 4-triazin derivative of diclofenac on changes in body weight in chlorpromazine induced Parkinson's rat

TREATMENT	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)
Group I (vehicle group)	240.0 ± 10.33	266.7 ± 10.22
Group II (Negative group)	240.0 ± 12.65	216.7 ± 10.54 a*
Group III (Standard group)	243.3 ± 11.16	256.7 ± 11.45 b*
Group IV (treatment group)	251.7 ± 12.49	280.0 ± 12.91 b***

Values are expressed as mean ± SEM, n=6,

Comparisons were made between a-Group I V_S II, III, IV b- Group II V_S III, and IV

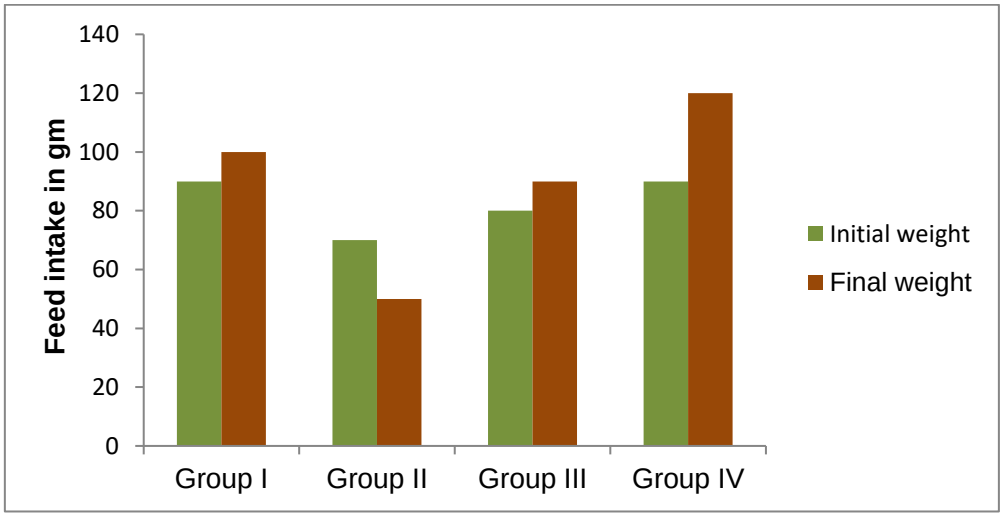
Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Graph No.: 1 Effect of 1, 2, 4-Triazin derivative of diclofenac on Changes in Body Weight in Chlorpromazine Induced Parkinson's Rat

Table No.: 7 Effect of 1, 2, 4-Triazin derivative of diclofenac on Feed Intake in Chlorpromazine Induced Parkinson's Rat

Treatment	Initial Feed Intake (g)	Final Feed Intake (g)
Group I (vehicle group)	90	100
Group II (Negative group)	70	50
Group III (Standard group)	80	90
Group IV (treatment group)	90	110



Graph No.: 2 Effect of 1, 2, 4-Triazin derivative of diclofenac on Feed Intake in Chlorpromazine Induced Parkinson's Rat

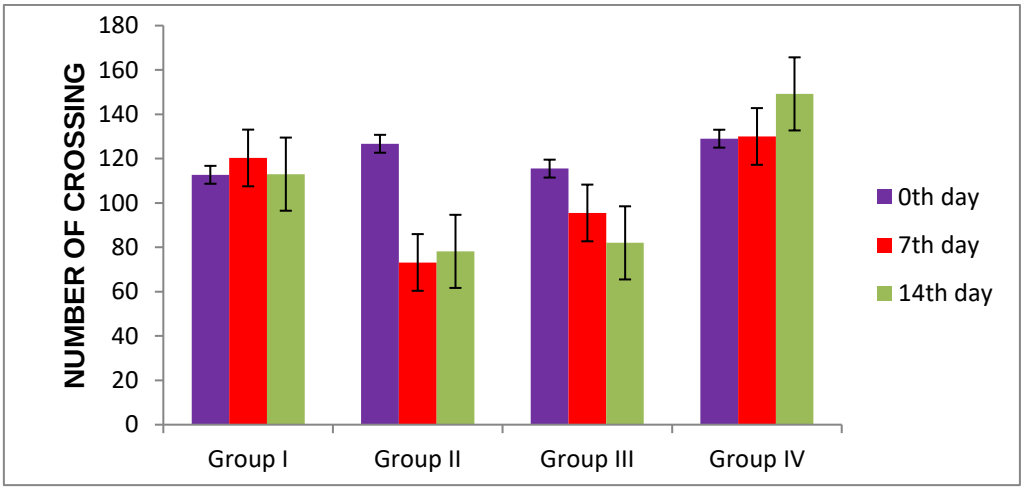
Table No.: 8 Effect of 1, 2, 4-Triazin derivative of diclofenac on Exploratory and Locomotors Activity by Using Actophotometer in Chlorpromazine Induced Parkinson's Rat

TREATMENT	0th day	7th day	14th day
GroupI (vehicle group)	112.7 ± 11.79	120.3 ± 12.21	113.0 ± 11.42
GroupII (Negative group)	126.7 ± 10.13	73.17 ± 6.45	78.17 ± 1.88a*
GroupIII (Standard group)	118.5 ± 11.53	95.50 ± 11.90a*	82.00 ± 4.68a*
GroupIV (treatment group)	129.0 ± 10.36	130.0 ± 12.48b*	149.2±11.71b***

Values are expressed as mean ± SEM, n=6

Comparisons were made between a-Group I Vs II, III, IV b- Group II Vs III, and IV

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Graph No.: 3 Effect of 1, 2, 4-Triazin derivative of diclofenac on Exploratory and Locomotors Activity by Using Actophotometer in Chlorpromazine Induced Parkinson's Rat

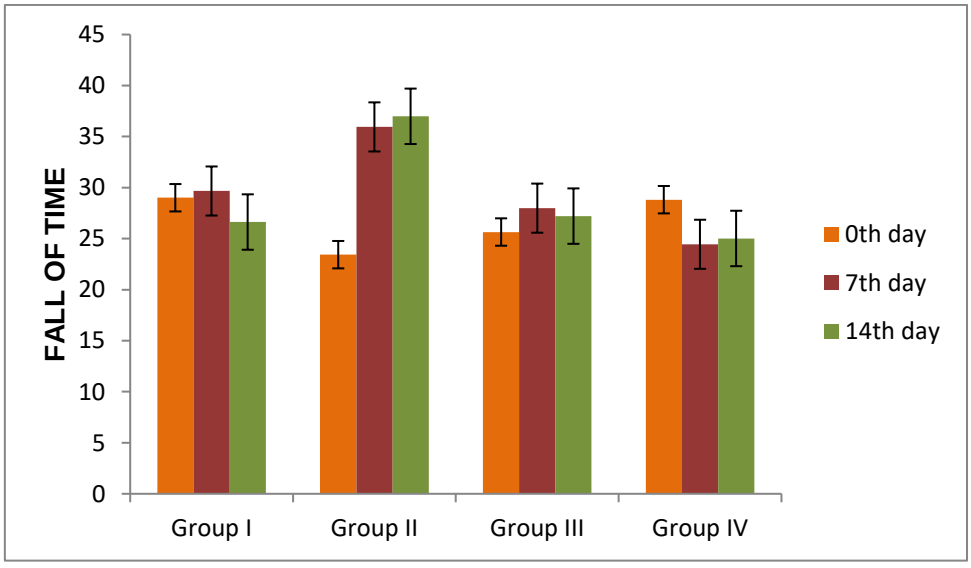
Table No.: 9 Effect of 1, 2, 4-Triazin derivative of diclofenac on Muscle Coordination Behaviour by Using Rota Rod in Chlorpromazine Induced Parkinson's Rat

TREATMENT	0th day	7th day	14th day
Group I (vehicle group)	116.7 ± 13.80	123.0 ± 10.78	138.7 ± 13.40
Group II (Negative group)	119.0 ± 11.62	91.0 ± 3.23a*	66.33±5.83a***
Group III (Standard group)	99.33 ± 7.41	124.2 ± 10.53	142.7 ± 11.50b***
Group IV (treatment group)	113.5 ± 10.29	125.0±11.00b***	140.7 ± 13.35b***

Values are expressed as mean ± SEM, n=6

Comparisons were made between a-Group I Vs II, III, IV b- Group II Vs III, and IV

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Graph No.: 4 Effect of 1, 2, 4-Triazine derivative of diclofenac on muscle coordination behaviour by using Rota Rod in chlorpromazine induced Parkinson's rat

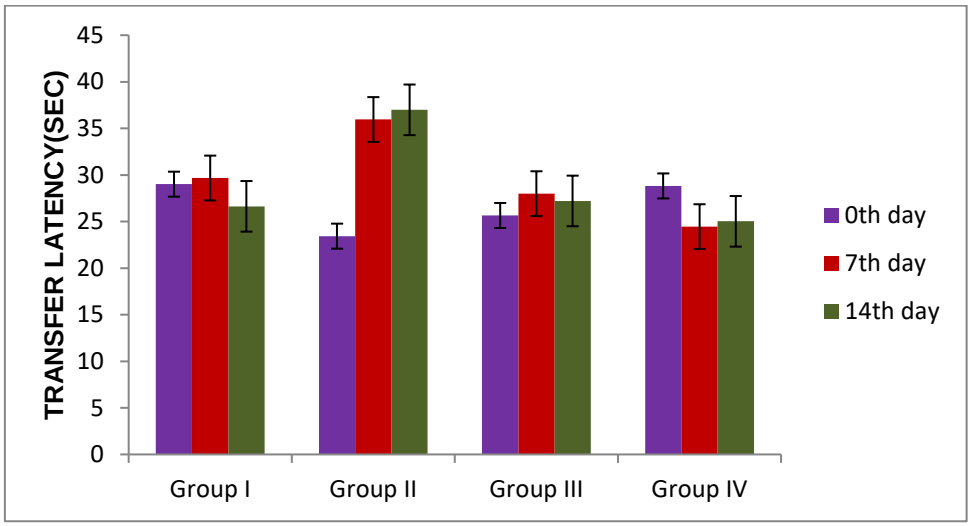
Table No.: 10 Effect of the 1, 2, 4-triazin derivative of diclofenac on cognitive performance by using morris water maze in chlorpromazine induced Parkinson's rat

TREATMENT	0th day	7th day	14th day
Group I (vehicle group)	29.01 ± 1.90	29.67 ± 1.88	26.63 ± 1.43
Group II (Negative control group)	23.43 ± 2.71	35.95 ± 1.27a*	36.99 ± 1.13a***
Group III (Standard group)	25.65 ± 1.51	27.99 ± 1.98b*	27.21 ± 1.91b***
Group IV (treatment group)	28.82 ± 1.60	24.45±1.25b***	25.02 ± 1.03b***

Values are expressed as mean ± SEM, n=6

Comparisons were made between a-Group I Vs II, III, IV b- Group II Vs III, and IV

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Graph No.: 5 Effect of the 1, 2, 4-Triazin derivative of diclofenac on Cognitive Performance by Using Morris Water Maze in Chlorpromazine Induced Parkinson's Rat

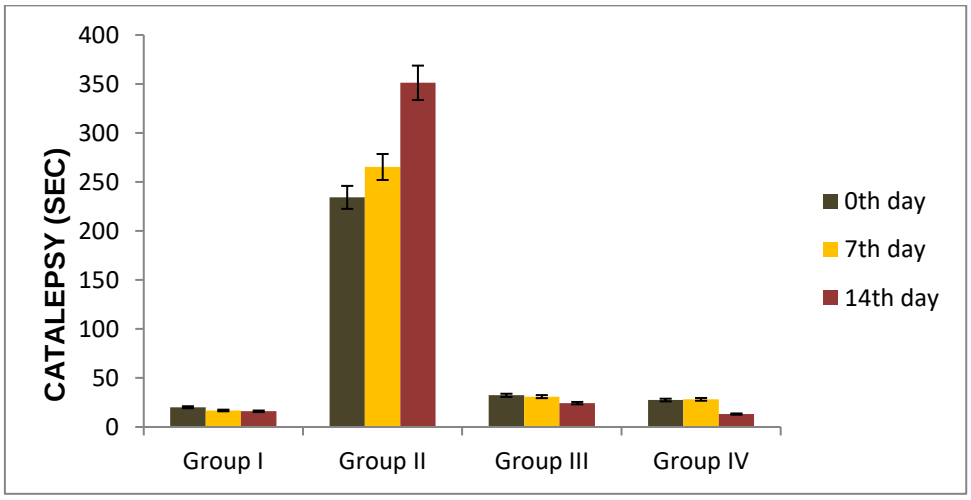
Table No.: 11 Effect of the 1, 2, 4-triazin derivative of diclofenac on catalepsy in chlorpromazine induced Parkinson's rat

TREATMENT	0th day	7th day	14th day
Group I (vehicle group)	20.17 ± 3.08	16.83 ± 1.77	16 ± 1.82
Group II (Negative control group)	234.3 ± 14.49	265.3±13.29a***	351.2 ± 15.46a***
Group III (Standard group)	32.33 ± 1.08	31.00 ± 1.96b***	24.33 ± 3.28b***
Group IV (treatment group)	27.50 ± 1.40	28.20 ± 1.67b***	13.17 ± 0.94b***

Values are expressed as mean ± SEM, n=6

Comparisons were made between a-Group I Vs II, III, IV b- Group II Vs III, and IV

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Graph No.: 6 Effect of 1, 2, 4-Triazin derivative of diclofenac on catalepsy in Chlorpromazine Induced Parkinson's Rat

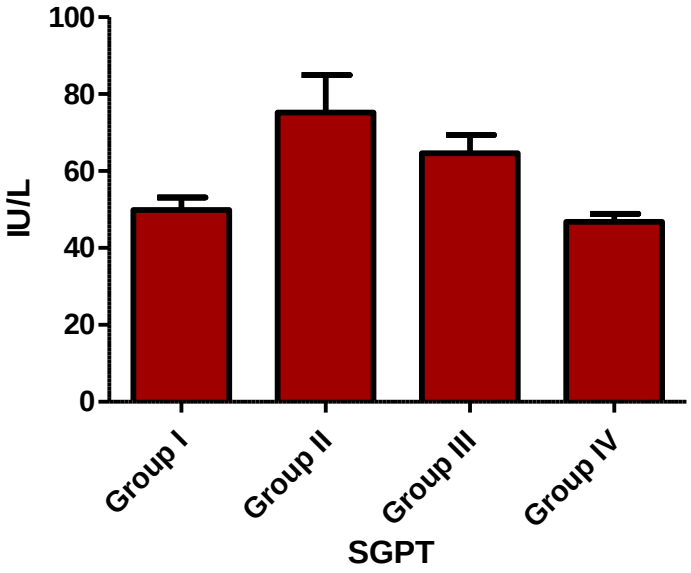
Table No.: 12 Effect of 1, 2, 4-triazine derivative of diclofenac on biochemical parameter in chlorpromazine induced Parkinson's rat

TREATMENT	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	BILIRUBIN (mg/dl)	Urea (mg/dl)	CREATININE
Group I (vehicle group)	49.89 ± 3.27	17.99 ± 2.92	80.25± 8.04	0.158±0.01	12.68±1.91	0.5117±0.07
Group II (Negative control group)	75.17 ±9.79^{a*}	38.24 ± 8.47^{a*}	142.7± 4.39^{a***}	0.681±0.08^{a***}	20.94±1.30^{a**}	0.9117±0.02^{a**}
Group III (Standard group)	64.64 ± 4.77	32.91 ± 4.05	97.32± 2.82	0.1967±0.02	20.24± 1.05^{a**}	0.805±0.07^{a*}
Group IV(treatment group)	46.80 ± 2.04^{b*}	17.30 ± 2.96^{b*}	60.73± 6.96^{a***}, ^{b***}	0.1317±0.01^{a***}, ^{b***}	12.74± 1.70^{b**}	0.4217±0.06^{b***}

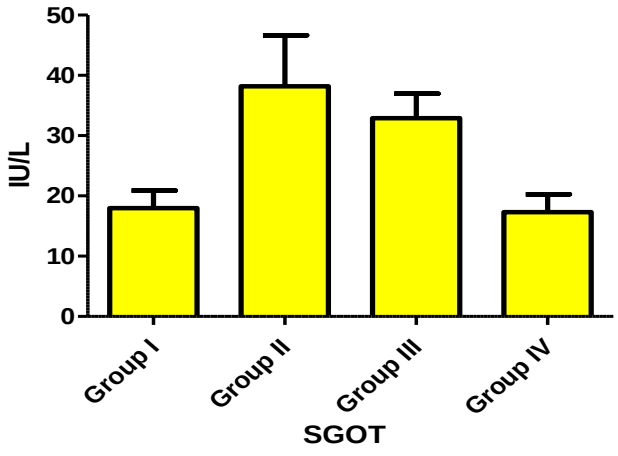
Values are expressed as mean ± SEM, n=6

Comparisons were made between a-Group I V_S II, III, IV b- Group II V_S III, and IV

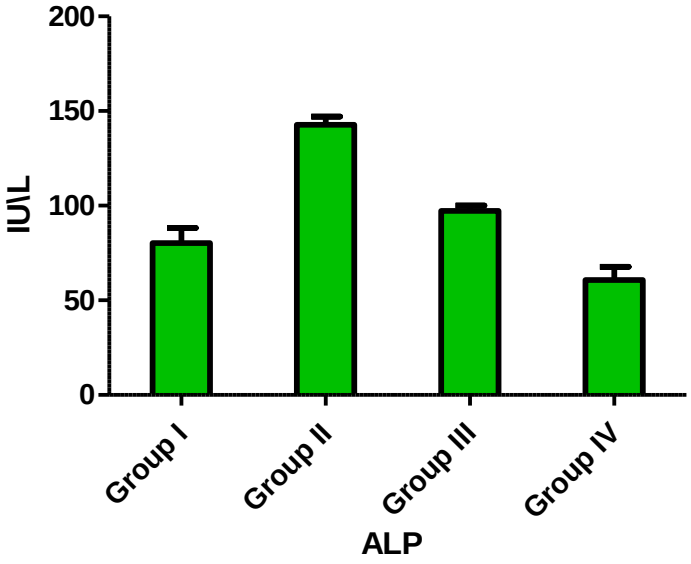
Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



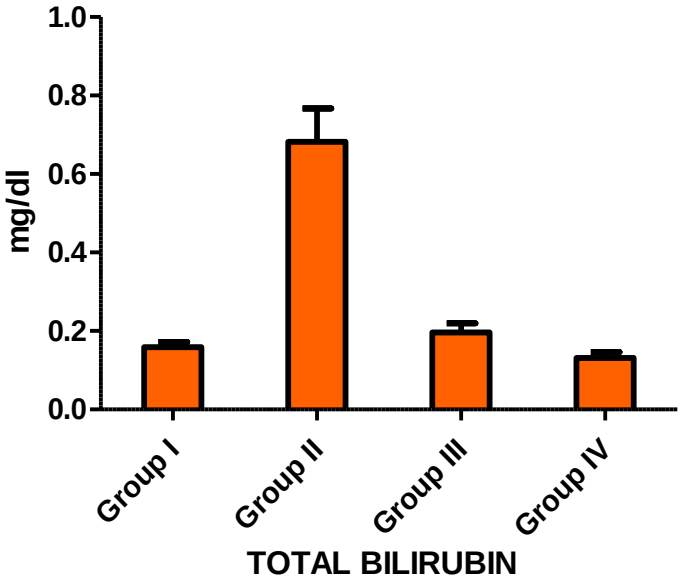
Graph No.: 7 Effect of 1, 2, 4-Triazine derivative of diclofenac on Serum Glutamate Pyruvate (SGPT) in chlorpromazine induced Parkinson's rat



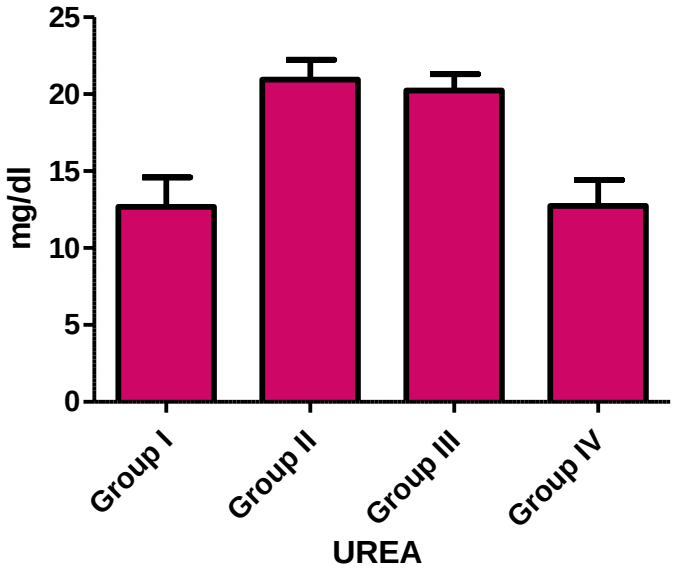
Graph No.: 8 Effect of 1, 2, 4-Triazine derivative of diclofenac on glutamate oxaloacetate transaminase (SGOT) in chlorpromazine induced Parkinson's rat



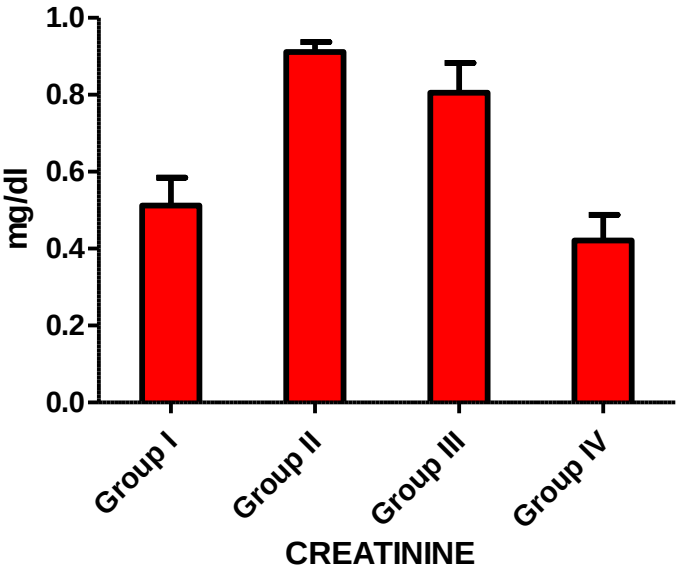
Graph No.: 9 Effect of 1, 2, 4-Triazine derivative of diclofenac on Alkaline phosphates in chlorpromazine induced Parkinson's rat



Graph No.: 10 Effect of 1, 2, 4-Triazine derivative of diclofenac on Total bilirubin in chlorpromazine induced Parkinson's rat



Graph No.: 11 Effect of 1, 2, 4-Triazine derivative of diclofenac on urea in chlorpromazine induced Parkinson's rat



Graph No.: 12 Effect of 1, 2, 4-Triazine derivative of diclofenac on Creatinine in chlorpromazine induced Parkinson's rat

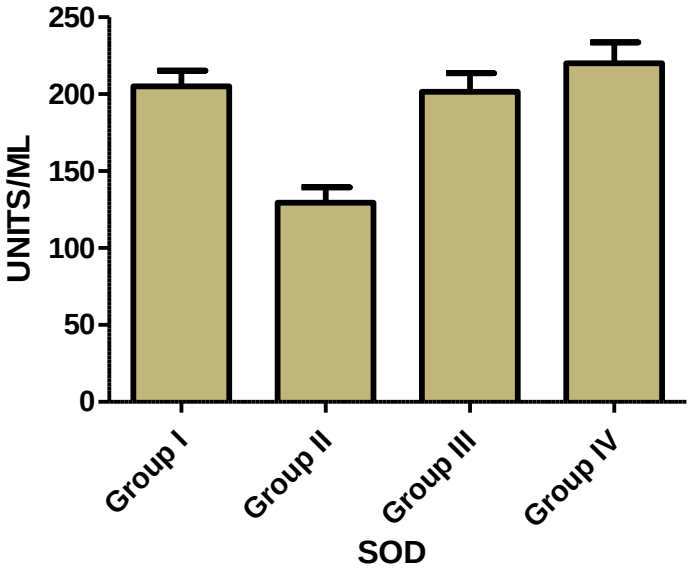
Table No.: 13 Effects of 1, 2, 4-triazin derivative of diclofenac on antioxidant enzyme levels in rat brain

TREATMENT	SOD (UNITS/ML)	GSH (Umol/L)	NITRITE (m mol/l)	PROTEIN (mg/dl)	LIPID PEROXIDASE (µmol\L)	CATALASE (mg/dl)	GLUTAMATE (mg/dl)
Group I (vehicle group)	205.2 ±10.22	4.417±0.23	3.670 ±0.25	0.6583± 0.05	1.665±0.18	272.8± 15.16	12.09± 0.82
Group II (Negative control group)	129.5 ±10.00^{a***}	2.003±0.21 a**	60.74 ± 3.82^{a**}	0.2050±0.05a***	2.336±0.22a *	189.8±12 .92a***	16.93± 1.08a**
Group III (Standard group)	201.5 ± 12.25^{b**}	5.877±0.69 a***,b***	6.280 ± 1.29a***	0.6733±0.05 a***	0.8133±0.27 a**, b***	243.3± 8.99b*	5.588± 0.83a***b***
GroupIV (treatment group)	220.0 ± 13.55^{b***}	8.028±0.43 a**	3.467 ± 0.74a***	0.8300±0.01 b***	1.152±0.36a ***, b***	327.8±5. 07a*,b***	3.577± 0.77a***

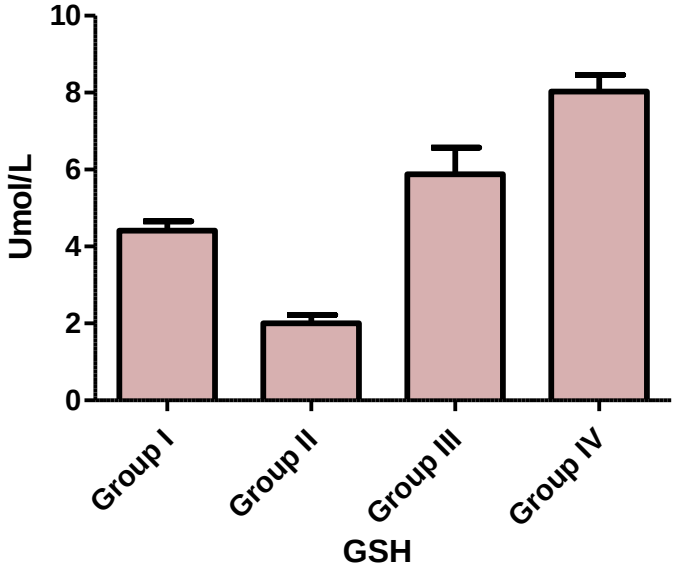
Values are expressed as mean ± SEM, n=6

Comparisons were made between a-Group I Vs II, III, IV b- Group II Vs III, and IV

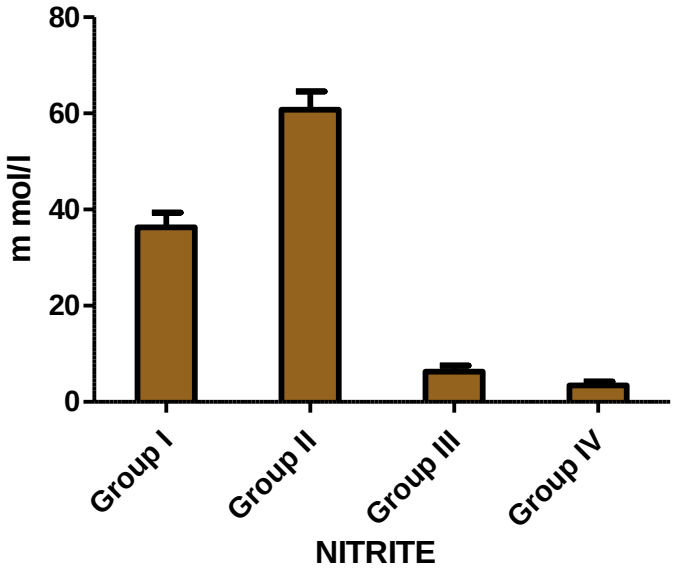
Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



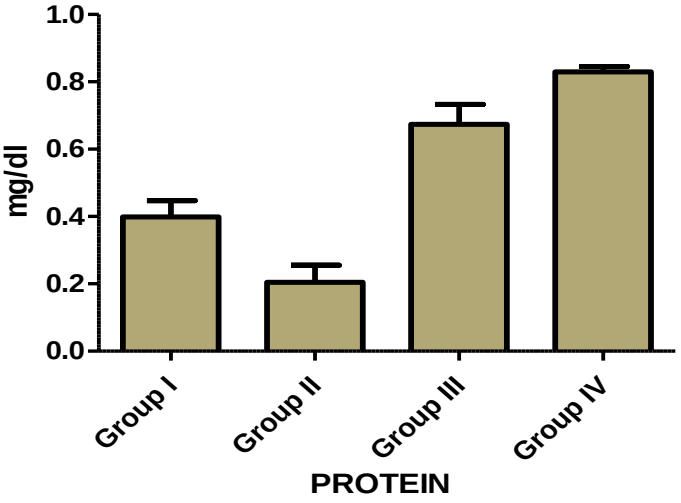
Graph No.: 13 Effect of 1, 2, 4-Triazine derivative of diclofenac on superoxide dismutase in chlorpromazine induced Parkinson's rat



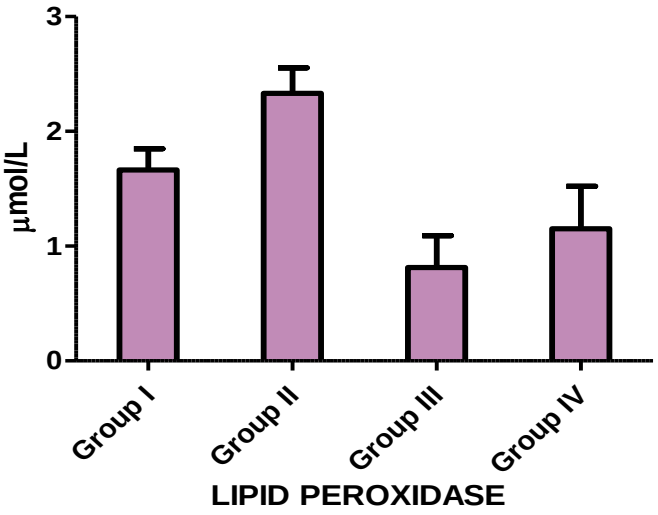
Graph No.: 14 Effect of 1, 2, 4-Triazine derivative of diclofenac on reduced glutathione (GSH) in chlorpromazine induced Parkinson's rat



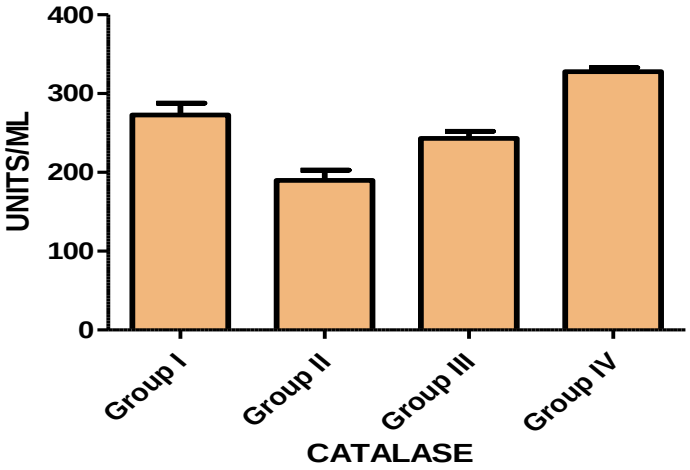
Graph No.: 15 Effect of 1, 2, 4-Triazine derivative of diclofenac on Nitrite in chlorpromazine induced Parkinson's rat



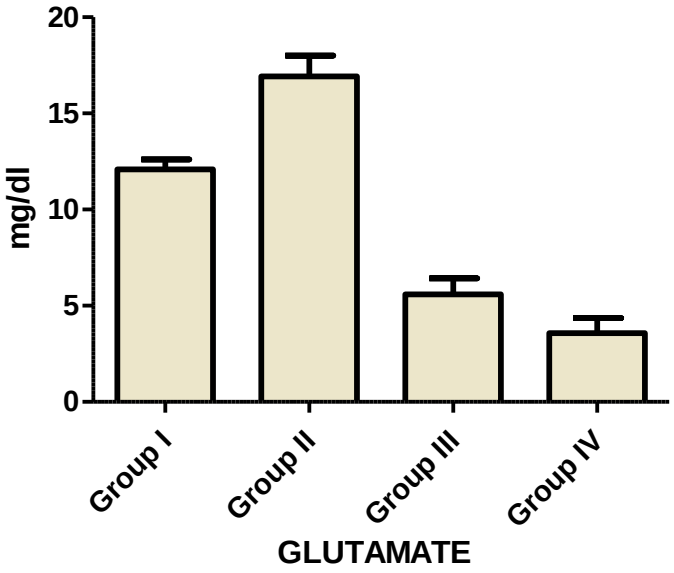
Graph No.: 16 Effect of 1, 2, 4-Triazine derivative of diclofenac on protein in chlorpromazine induced Parkinson's rat



Graph No.: 17 Effect of 1, 2, 4-Triazine derivative of diclofenac on Lipid Peroxidase in chlorpromazine induced Parkinson's rat



Graph No.: 18 Effect of 1, 2, 4-Triazine derivative of diclofenac on Catalase in chlorpromazine induced Parkinson's rat



Graph No.: 19 Effect of 1, 2, 4-Triazine derivative of diclofenac on glutamate level in chlorpromazine induced Parkinson's rat

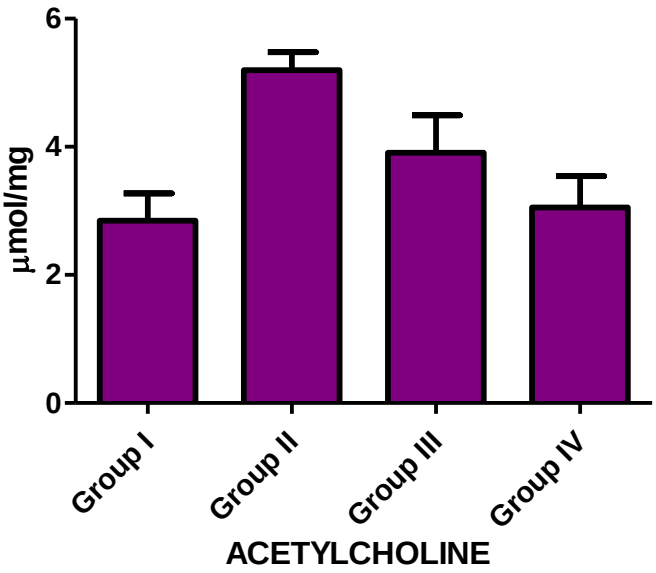
Table No.: 14 Effects of 1, 2, 4-Triazin Derivative of diclofenac on Brain Ach Levels in Rat Brain

Values are expressed as mean ± SEM, n=6

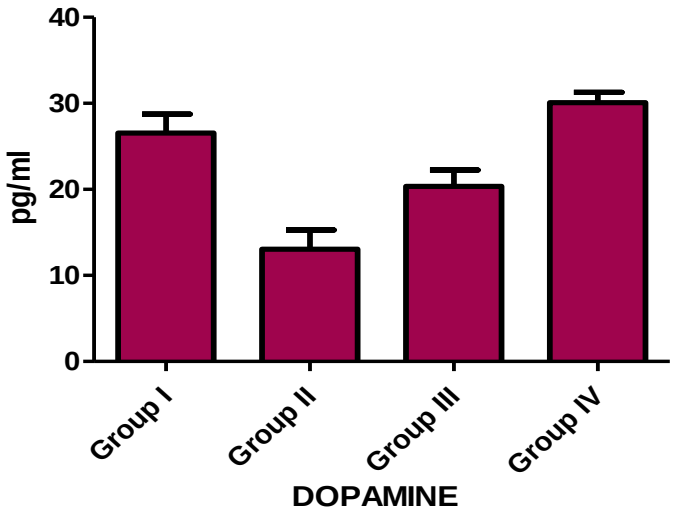
Comparisons were made between a-Group I Vs II, b- Group II Vs IV

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05

TREATMENT	ACETYLCHOLINE (µmol/mg)	DOPAMINE (pg/ml)
Group I (vehicle group)	2.848 ± 0.42	26.34 ± 2.20
Group II (Negative control group)	5.197 ± 0.28^{a**}	13.04 ± 2.24^{a***}
Group III (Standard group)	3.907 ± 0.58	20.36 ± 1.89^{b***}
Group IV (treatment group)	3.052 ± 0.48^{b*}	30.10 ± 1.19^{b**}



Graph No.: 20 Effects of 1, 2, 4-Triazin Derivative of diclofenac on Brain Ach Levels in Rat Brain



Graph No.: 21 Effect of 1, 2, 4-Triazin Derivative of diclofenac on Dopamine Assay in Chlorpromazine Induced Parkinson's Rat

CHAPTER- 8

DISCUSSION

CHAPTER- 8

DISCUSSION

NSAIDs are the most common medication used in the neuro inflammatory disorders. Among that diclofenac is one of the prominent drugs which used in Parkinson's. Diclofenac elicit appreciable GI irritation, bleeding and ulceration produced. Synthetic approaches based upon chemical modification of Diclofenac have been taken with the aim of improving safety profile and in turn therapeutic window. Carboxylic group is a major reason for the GI toxicity of Diclofenac, so Structural replacement of carboxyl group may reduce the GI toxicity. 1, 2, 4-Triazine derivative as antagonists of Adenosine A_2 receptor is expressed in the basal ganglia where it functionally opposes the actions of the dopamine D2 receptor. i.e., inhibition of the A_2 receptor leads to enhancement of D2 receptor function. Previous study reported that 1, 2, 4-Triazin derivative may possess appropriate Anti Parkinson's action [13]. Hence in this study carboxyl group of diclofenac was replaced by addition of 2- chloroacetamide to produce 1, 2, 4-Triazin derivative of diclofenac.

In this the intermediate and the final 1, 2, 4-Triazin derivative of diclofenac was confirmed by IR spectra region used to identification the functional group. The compounds showed the possible peak of IR (KBr), v, cm-1: CH, C=O, C-O-H, N-H, C-N, CH_2, C-Cl shows the presence of the functional groups.

Molecular docking of 1, 2, 4-Triazin derivative of diclofenac was analysed against 5 major targets. The Structure of 1, 2, 4- Triazin derivative of Diclofenac was drawn by using chem. office 2004 software and docking simulation was carried out against the Parkinson's enzyme targets like 1) Dopamine receptor D3 protein (3PBL),2) Dopa decarboxylase-DDC (1JS3), 3) Adenosine A2 receptor-AA2AR (3EML), 4) P38 map kinase (2ZAZ), 5) Monoamino oxidase-B-MAO-B (2V5Z) enzymes target with the help of Autodock k4 program. In this study, MAO-B , MAPK shows high binding site involved in this target and D_3 protein, DDC, AA2AR, 1JS3, 3PBL shows slightly low binding site when compare to the MAO-B, MAPK PD which increased the binding abilities of molecular docking scores of designed ligand.

The 1, 2, 4-Triazin derivative compound not produced any toxic symptoms or mortality up to the dose level of 300mg/kg orally in mice and hence, the drugs were considered safe for further pharmacological screening. Up to 14 days Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin; fur, eyes and mucous membranes, and also cardio vascular system, central nervous systems, autonomic nervous and gastro intestinal tract in the animals, during the period test compound do not produce any major toxicity. Hence, the 1, 2, 4-Triazin derivative synthesized drug was selected as *1/10* of the *dose* that was used in the further *pharmacological screening.* Selected dose for *in vivo* study was 30 mg/kg.

Induction of Parkinson's disease in the rat was carried out by administration of chlorpromazine 3mg/kg, (dissolved with 1% gum acacia in distilled water suspension) by intra peritoneal route. Chlorpromazine drug was centrally acting with high affinity for dopamine D2 receptors antagonists [90].

In PD condition reduction in the body weight during the study period development may be due to hypothalamic regulation, energy expenditure, or dopaminergic signaling mechanism [91]. In treatment with 1, 2, 4-Triazine derivative group shows significantly increase in body weight as compare to Diclofenac group and chlorpromazine group.

In this study PD condition caused decreased in feed intake and BMI variation which may reflect a dysregulation of dopaminergic control of eating behavior rather than modification of energy metabolism [92]. The standard groups shows significantly increased, feed intake when compared to chlorpromazine group and the 1, 2, 4-Triazin treatment group are showed significantly increased feed intake when compared to standard group.

Actophotometer was used to evaluate the effect of locomotors activity improvement properties of rat. Chlorpromazine induced group were exhibited CNS depressant effect due to impaired cholinergic transmission, oxidative / nitrergic stress, neuroinflammation, and dyslipidemia [93]. In this study diclofenac treated group were showed no significant impact on locomotion when compared to the PD control. The 1, 2, 4-Triazin derivative of diclofenac treated group shows

significantly improved spontaneous locomotor activity hence indicating its CNS stimulant activity in Parkinson's rats when compared to PD control group.

Muscle coordination activity of chlorpromazine induced rat was significantly decreased because in the Sensory fibers detect lengthening of the muscles and cause motor neurons in the spinal cord to contract the same muscles [94]. In this study the treatment group diclofenac and 1, 2, 4-Triazin derivative of diclofenac showed significantly increased in muscle coordination when compared to the PD control.

In this study, MWM test on the manifested itself due to immersion stress, fatigue and sensory-motor deficits showed in chlorpromazine Group shows significantly decreased [95]. Cognitive function was significantly increased in both the treatment groups when compared to chlorpromazine group. In comparison between the treatment groups 1, 2, 4 Triazin group shows more increased in cognitive function when compare to Diclofenac Group.

In PD condition, Catalepsy has as inability to correct an imposed abnormal posture while maintaining the righting reflex due to its non-selective action, it also produces blockade of post-synaptic D_2 receptors in the nigrostriatal pathway leading to the development of extra pyramidal side effects. In 1, 2, 4-Triazin derivative treatment Group shows significantly increased in posture correction when compared to Diclofenac group.

In this study, serum SGPT, SGOT, ALP, Total bilirubin, was raised in Parkinson's disease rat due to liver cells damage [96]. In the Diclofenac treatment and 1, 2, 4-Triazin treatment group shows significantly low level of liver enzymes in serum. It reveals that the non toxic nature of diclofenac and synthesized compound on the liver when compared to the chlorpromazine treated Group.

In PD condition Creatinine and urea was increased due to impaired renal functions evidence by an increase in serum urea and creatinine concentration [97]. Treatment group shows significant decrease in serum creatinine and urea as compare to the PD control. It reveals that non toxic nature of diclofenac and synthesized compound on kidney when compared to the chlorpromazine treated Group.

Brain Superoxide dismutase (known as SOD) is an enzyme which acts as a catalyst in the process of dismutation of superoxide into nonreactive oxygen species and hydrogen peroxide. It is therefore a critical antioxidant defense which is present in nearly all cells which are exposed to oxygen. Superoxide dismutase helps in neutralizing the toxic effects of free radicals [98]. In this study, Diclofenac group showed significantly increases in SOD level when compared to chlorpromazine group. The 1, 2, 4-Triazin derivative treatment group shows brain protects against oxidation stress by significantly increases in SOD level when compared to chlorpromazine group.

In this study, chlorpromazine group shows GSH depletion and reduced glutathione in the substantia nigra in Parkinson's disease could be the result of neuronal loss. As a matter of fact, the positive correlation has been found to exist between the extent of neuronal loss and depletion of glutathione. A decrease in the availability of reduced glutathione would impair the capacity of neurons to detoxify hydrogen peroxide and increase the risk of free radical formation and lipid peroxidation [99]. The treatment group shows significantly increases in GSH level when compared to PD control group.

In PD condition significantly increase due to nitric oxide has been involved in the cytotoxicities by activation of macrophages or excess stimulation of neurons by glutamate [100]. Diclofenac group shows significantly decrease in nitrite level when compared to chlorpromazine group. Treatment group shows significantly decrease in nitrite level when compared to PD control group

In Chlorpromazine group shows significantly decrease in brain protein level when compared to control group. Treatment group shows significantly increase in protein level when compared to chlorpromazine alone treated group.

Lipid peroxidation, a sensitive marker of oxidative stress, was estimated by measuring the levels of TBA. It is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function, impaired structural integrity, decreased fluidity, and inactivation of number of membrane bound enzymes [101]. In this study, Treatment group shows significantly decrease in lipid peroxidation level when compared to chlorpromazine group.

Chlorpromazine group shows significantly decrease due to catalase enzyme accumulation of precursor to free radical biosynthesis [102]. In this study, treatment group shows significantly increase in catalase level when compared to control group. And then Diclofenac group and chlorpromazine group shows significant decreases in TBA when compared to Diclofenac group.

Chlorpromazine group shows significantly increase in brain glutamate level due to increased density of dopamine receptor and decreased levels of dopamine is observed in chlorpromazine treatment [103]. Treatment group shows decreased density of dopamine receptor by significantly decreases the glutamate level when compared to Chlorpromazine group.

In this study, brain acetylcholine increase significantly in chlorpromazine alone treated group when compared to control group. 1, 2, 4-Triazin derivative shows significantly decreased in acetylcholine level when compare to chlorpromazine group. Diclofenac group showed no significant difference when compared to control group [104].

The dopamine level in chlorpromazine induced rats was reduced significantly when compared to control group. Treatment groups have high dopamine level indicate recovering neurodegeneration.

In ulcerogenecity Chlorpromazine Group shows mild ulceration when compared to control group. Diclofenac produce more ulceration when compared to control. In 1, 2, 4-Triazin derivative treated group shows very less incident of ulceration when compare to diclofenac and chlorpromazine treated group [105].

Histopathology of mid brain portion of brain section of parkinson's rat showed hyperchromatic nuclei with eosinophilic vacuolated cytoplasm in edematous. In treatment group shows hyperchromatic nuclei and mild vacuolization suggestive of mild degenerative changes in treated animals [106]. It conforms the diclofenac and 1, 2, 4-Triazin derivative treatment shows greater effect on neuronal inflammation.

CHAPTER - 9

SUMMARY AND CONCLUSSION

CHAPTER - 9

SUMMARY AND CONCLUSSION

Parkinsonism disease (PD) is neurodegenerative disorder, is characterized by progressive loss of dopamine (DA) neurons in the substantial nigra pars compacta (SNpc), leading to striatal DA depletion. This degeneration of Dopaminergic nigrostriatal system is largely responsible for the classical motor signs, including resting tremor, muscle rigidity, and bradykinesia. Molecular docking study of 1, 2, 4 -Triazin Analogue of diclofenac shows greater interaction against MAO-B and MAPK as compared to the D3 protein, DDC and $AA_{2A}R$ targets.

In this study the carboxylic group of diclofenac was replaced with 2-Chloroacetamide to produce the 1, 2, 4 - Triazin derivative of diclofenac as potential target for PD. The functional groups are confirmed by IR spectra functional group peaks.

PD was induced in this study by administration of chlorpromazine due to its D2 dopamine receptor blockade action. It confirms the decreased locomotors function, muscle coordination, cognitive function and deflects of dopamine, also increased in muscle rigidity, oxidative stress and cholinergic activity in the chlorpromazine alone administered group.

Diclofenac and 1, 2, 4 - Triazin derivative of diclofenac treatments significantly revert all this complication of PD. But the ulcerogenisity was significantly higher in diclofenac treated group on continuous 14 day treatment it might be due to its carboxyl group and the treatment of 1, 2, 4 - Triazin derivative of diclofenac posses more potent anti-parkinson activity with negligible ulcer as compared to the diclofenac treatment. It might be due to the replacement of carboxyl group of diclofenac with 2-chloroacetamide and also the potent action of 1, 2, 4 triazine against PD.

In conclusion replacement of carboxyl group of diclofenac with 2-chloroacetamide to produce a 1, 2, 4 triazine analogue as a potent ligand for parkinson’s disease with minimal GI toxicity. Further clinical data are required to

explore this synthesised Analogue of Diclofenac as Potential Ligand for improving the status of PD patients.

CHAPTER-10

BIBLIOGRAPHY

CHAPTER-10

BIBLIOGRAPHY

1. Santiago Perez-Lloret, Marıa Veronica Rey, Estelle Dellapina, Jean Pellaprat, Christine Brefel-Courbon & Olivier Rascol. Emerging analgesic drugs for Parkinson's disease. Expert Opin. Emerging Drugs 2012 17(2):157-171.
2. Lang A.E, Lozano A.M. Parkinson;s diseases (part -1 and part 2) New England. Journal of medicine 339, 1998, 1044-1052, 1130-1143.
3. Karen Rees, Rebecca Stow, Smitaa Patel, Natalie Ives, Kieran Breen, Carl E Clarke, Yoav Ben-Shlomo (2011). Non-steroidal anti-inflammatory drugs as disease-modifying agents for Parkinson's disease: evidence from observational studies. Cochrane Database of Systematic, Issue 11. Art. No.: CD008454.
4. Lorraine V Kalia. Seminar on Parkinson's disease. Vol-386. Lancet; 2016 ,386: 896–912.
5. George DeMaagd. Parkinson's disease and Its Management. Disease Entity, Risk Factors, Pathophysiology, Clinical Presentation, and Diagnosi. P&T®. 2015, Vol. 4
6. YoungSoo Kim. Pathology of Neurodegenerative Diseases, Brain Damage - Bridging Between Basic Research and Clinics, Dr. Alina Gonzalez-Quevedo (Ed.), 2012, ISBN: 978-953-51-0375-2.
7. Adriana Galvan and Thomas Wichmann.Pathophysiology of Parkinsonism. Clin. Neurophysiol; 119(7):2008, 1459–1474.
8. Timothy R. Mhyre, James T. Boyd, Robert W. Hamill and Kathleen A. Maguire-Zeiss. Parkinson's diseases. Subcell Biochem;2012,65: 389–455.
9. Irmina Urbanek, Krzysztof Kaczmarek, Iwona Cygankiewicz, Pawel Ptaszynski. Risk-benefit assessment of ivabradine in the treatment of chronic heart failure. Volume 2014:6 Pages 47—54.
10. Jeremy N. Cashman. The Mechanisms of Action of NSAIDs in Analgesia. Volume 52, 1996, pp 13–23.
11. Bacchi s, Palumbo P, Sponta A and Coppolino M F. Clinical Pharmacology of Non-Steroidal Anti-Inflammatory Drugs: A Review. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry 11, 2012, 52-64.

12. Sadaf Naeem, Rahila Njam, Nousheen Alam. antidepreeseant effect of diclofenac against Experimental parkinson's rat model. American Journal of Pharmacological Science Vol.3 .No.3.2015, 74-78.
13. Garvey D S, Glavin A ET. Al. Nitrosothiol esters of diclofenac: synthesis and pharmacological characterization as gastrointestinal-sparing prodrugs. J.Med. chem., 43, 2000, 4005-4016.
14. Miles Congreve, Stephen P. Andrew, et al. Discovery of 1, 2, 4-Triazine derivatives as adenosine A2A antagonists using structure based drug design. J. Med. Chem, 55, 2012 1898-1903.
15. Wermuth C G.Selective optimization of side activities: Another way for drug discovery. J.Med che, 47, 2004, 1303-1314.
16. Bosquesi, P L, Melo, T R F, Vizioli, E O, Santos, J L, Chung M C. Anti-inflammatory drug design using a molecular hybridization approach, pharmaceutical, 4, 2011, 1450-1474.
17. Halen, P K, Murumkar, P R, Giridhar R, Yadav M R. Prodrug designing of NSIDs mini rev.med.chem, 9, 2009, 24-139.
18. Gabor G. Kovacs. Neuropathology of neurodegenerative diseases. Cambridge university press. 2015, ISBN 978-1-107-58866-0.
19. Serge Przedborski, Miquel Vila, and Vernice Jackson-Lewis. Neurodegeneration: What is it and where are we? The Journal of Clinical Investigation, Vol. 111, 2003, 111:3–10.
20. Jellinger K A. Recent developments in the pathology of Parkinson's disease. J Neural Transm Suppl. 2002;(62):347-76.
21. Sergio Rosales-Mendoza and Dania O Govea-Alonso. The potential of plants for the production and delivery of human papillomavirus vaccines. Expert Rev. Vaccines Early online, 1–11 (2015)
22. Mauro Fasano, Chiara Monti and Tiziana Alberio. Review on A systems biology-led insight into the role of the proteome in neurodegenerative diseases. Expert review of proteomics, vol. 13, 2016 NO. 9, 845–855.
23. Jaime I Arevalo-Villalobos, Sergio Rosales-Mendoza, Sergio Zarazu. Immunotherapies for neurodegenerative diseases: current status and potential of plant-made biopharmaceuticals. Journal: Expert Review of Vaccines. Volume 16, 2017, Issue 2 .

24. Frans g I. Jennekens neurowords A Short History of the Notion of Neurodegenerative Disease. Journal of the History of the Neurosciences, (2014). 23:85–94, 2014.
25. Saumitra Basu. History of Neurodegenerative Diseases and its Impact on Aged Population in India: An Assessment. Indian Journal of History of Science, 52.1, 2017.106-115.
26. Maja relja, Pathophysiology and classification of neurodegenerative diseases. Ejifcc: www.ifcc.org/ejifcc. 2014, Vol 15 no 3.
27. Kurt A. Jellinger. Interaction between α -Synuclein and Other Proteins in Neurodegenerative Disorders. TheScientificWorldJOURNAL. Volume 11 (2011), Pages 1893-1907.
28. Kurt a. Jellinger. Criteria for the neuropathological diagnosis of dementing disorders: routes out of the swamp?. recent researches in modern medicine, 2010, isbn: 978-960-474-278-3.
29. Kovacs. Neuropathology of neurodegenerative diseases. Cambridge university press. 2015, ISBN 978-1-107-58866-0.
30. Yahya E. Choonara, Pradeep Kumar, Girish Modi and Viness Pillay (2016) Publisher: Taylor & Francis. Journal: Expert Opinion on Drug DeliveryImproving drug delivery technology for treating neurodegenerative diseases. Volume 2016, Article ID 9417874, 19 pages.
31. Dena G. Hernandez, xylena reed and Andrew B. singleton. Review of Genetics in Parkinsonism disease: mendelian versus non-mendelian inheritance. J. Neurochem. 139 (Suppl. 1), 2016, 59—74.
32. Robbins and cotran. pathologic basis of disease 7th edition, Elsevier. 2008, ISBN 978-0-8089-2302-2.
33. Mar VIN M. Goldenberg. Medical Management of Parkinson's disease. P&T®. 2008, Vol. 33 No. 10.
34. JoaoMassano and Kailash P. Bhatia. Clinical Approach to Parkinson's disease: Features, Diagnosis, and Principles of Management. Cold Spring Harb Perspect Med; 2012, 2:a008870.
35. Jankovic j. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 2007, 79:368–376.
36. Douglas J. Gelb, Eugene Oliver, Sid Gilman. Diagnostic Criteria for Parkinson Disease.arch neurol / 1999, vol 56.

37. Olanow C W and Tatton W G. Etiology and pathogenesis of parkinson's disease. Annu. Rev. Neurosci. 1999, 22:123–44.
38. Anthony H. Schapira,, FMedSci, and Peter Jenner. Review of Etiology and Pathogenesis of Parkinson's disease. Movement Disorders, 2011, Vol. 26, No. 6.
39. Garrett E. Alexander., Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. Dialogues Clin Neurosci. 2004; 6:259-280.
40. Goodman and Gilman's. The pharmacological basis of therapeutics. International edition 2001, ISBN 0-07-112432-2.
41. Christopher G. Goetz. The History of Parkinson's disease: Early Clinical Descriptions and Neurological Therapies Cold Spring Harb Perspect 2011, 1:a008862.
42. Joshua M. Shulman, Philip L. De Jager, and Mel B. Feany. Parkinson's disease: Genetics and Pathogenesis. Annu. Rev. Pathol. Mech.2011, Dis.6:193-222.
43. Ole-Bjørn Tysnes, Anette Storstein. Neurology and preclinical neurological studies - review article. J Neural Transm. 2017, 15(4):473–490.
44. Dr. Daniela Berg, Dr. Ronald B. Postuma, Review of MDS Clinical Diagnostic Criteria for Parkinson's disease. Movement Disorders, Vol. 30, 2015, No. 12.
45. Christopher Frank, Giovanna Pari, John P. rossiter, Approach to diagnosis of Parkinson disease. Can Fam Physician; 2006, 52: 862-868.
46. Amos D. Korczyn pharmacological aspects of Drug treatment of Parkinson's disease. Dialogues in Clinical Neuroscience - Vol 6. 2004. No. 3.
47. Joseph Jankovic, L Giselle Aguilar (2008). Current approaches to the treatment of Parkinson's disease. Neuropsychiatric Disease and Treatment : 4(4) 743–757.
48. Harald E Vonkeman and Mart AFJ van de Laar. Cardiovascular case fatality in rheumatoid arthritis is decreasing; first prospective analysis of a current low disease activity rheumatoid arthritis cohort and review of the literature. BMC Musculoskeletal Disorders201415:142

49. Tracy S. Hunter, Chip Robison and Philip P. Gerbino. Emerging Evidence in NSAID Pharmacology: Important Considerations for Product Selection | Page 3. Drug Saf. 2012; 35(12):1127-1146.
50. Carlos Sostres, Carla J. Gargallo, Maria T. Arroyo, et.al. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Practice & Research Clinical Gastroenterology 24, 2010, 121–132
51. Vandekerckhove S, Müller C, Vogt D, Lategan C, Smith PJ, Chibale K, De Kimpe N, D'hooghe M. (2013) Synthesis and antiplasmodial evaluation of novel (4 aminobutyloxy) quinolines. Bioorg Med Chem Lett. 23(1): 318-22.
52. Roy Altman, Bill Bosch, Kay Brune, Paola Patrignani, and Clarence Young (2015). Advances in NSAID Development: Evolution of Diclofenac Products Using Pharmaceutical Technology. Clarence Young, 75:859–877.
53. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat N Biol.; 1971, 231(25):232–5.
54. Selg E, Buccellati C, Andersson M, et al. Antagonism of thromboxane receptors by diclofenac and lumiracoxib. Br.J.Pharmacol; 2017,152:1185-95.
55. Whelton A. Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiologic foundations and clinical implications. Am J Med;1999, 106:13S-24S
56. Silverstein FE, Faich G, Goldstein JL, et al. Gastro intestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA; 284:1247-55.
57. Dilger K, Herrliinger C, Peters J, et al.Effects of celecoxib and diclofenac on blood pressure, renal function, and vasoactive prostanoids in young and elderly subjects. J Clin Pharmacol; 2002, 42:985-94.
58. Zimmerman HJ. Update of hepatotoxicity due to classes of drugs in common clinical use: non-steroidal drugs, anti-inflammatory drugs, antibiotics, antihypertensives, and cardiac and psychotropic agents. Semin Liver Dis; 1999, 10: 322-38.

59. Zhang W, Moskowitz RW, Nuki MB, et al. OARSI recommendations for the management of hip and knee osteoarthritis, part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage; 2008, 16:137-62.
60. Rainsford KD, Kean WF, Ehrlich GE. Review of the pharmaceutical properties and clinical effects of the topical NSAID formulation diclofenac epolamine. Curr Med Res Opin; 2008, 24:2967-92.
61. Tong J. Gan. Diclofenac: an update on its mechanism of action and safety profile. Current Medical Research & Opinion Vol. 26,2 010 No. 7, 2010, 1715–1731.
62. Sivaraj S, Muthumani P, Venkataraman S, Raamamurthy J, Siva kumar R and Kumarnallasivan P. Design, molecular docking, synthesis and evaluation of some novel heterocyclic analogues of diclofenac as potent analgesic and anti-inflammatory agents with less ulcerogenicity. Der Pharma Chemica,2012, 4(3):1339-1349. ISSN 0975-413X.
63. Rumpa Banerjee, Riya Adhya and Abhimanyu Thakur. Improvement in binding affinity of Ginkgolide B in comparison to Levodopa: A molecular docking Study. J. Chem. Pharm. Res., 8(1):729-732,2016, ISSN: 0975-7384.
64. Faizul Azam, Najah Mohamed & Fatma Alhussen. Molecular interaction studies of green tea catechins as multitarget drug candidates for the treatment of Parkinson's disease: computational and structural insights. Network: Computation in Neural Systems.2016, ISSN: 0954-898X.
65. Miles Congreve, Stephen P. Andrews, Andrew S. Dore, Kaspar Hollenstein, Edward Hurrell, Christopher J. Langmead, Jonathan S. Mason, Irene W. Ng, Benjamin Tehan, Andrei Zhukov, Malcolm Weir, and Fiona H. Marshall.Discovery of 1, 2, 4-Triazine Derivatives as Adenosine A2A Antagonistsusing Structure Based Drug Design. Med. Chem.2012, 55, 1898−1903.
66. Souravh bais, GILL.S G and nitan kumar. Neuroprotective effect of juniperus communis on chlorpromazine induced Parkinson disease in animal model.2015, ID 542542, 7.
67. Karpakavalli M, Sivakumar T, Kumar .E.P and Nagaraja Perumal G . Evaluation of anti inflammatory activity of synthetic 7-flavanol derivatives. Der Pharma Chemica,2013, 5(2):154-156. ISSN 0975-413X.

68. OECD -423 (2001). OECD guideline for testing of chemicals. Acute Oral Toxicity – Acute Toxic Class Method.
69. Reenu Jacob, Nalini G and Chidambaranathan N. Neuroprotective effect of Rhodiola rosea Linn against MPTP induced cognitive impairment and oxidative stress. Annals of neurosciences, 2013, volume 20, number 2.
70. Yah-se K. Abada, Huu Phuc Nguyen, Rudy Schreiber, Bart Ellenbroek (2013).Assessment of Motor Function, Sensory Motor Gating and Recognition Memory in a Novel BACHD Transgenic Rat Model for Huntington Disease. PLoS ONE 8(7): 2013, e68584.
71. Uma Bhosale, Radha yegnanarayan, Pophale Prachi, Mandar Zambare, R.S.Somani. Study of CNS depressant and behavioral activity of an ethanol extract of Achyranthes Aspera (Chirchita) in mouse model. Annals of neurosciences, 2011, Volume 18 Number 2.
72. Alvin V. Terry Jr, Samantha E. Warner, Leah Vandenhuerk, Anilkumar Pillai, Sahebarao. Negative Effects of Chronic Oral Chlorpromazine and Olanzapine Treatment on the Performance of Tasks Designed to Assess Spatial Learning and Working Memory in Rats. Neuroscienc; 2008, 156(4): 1005–1016.
73. Joseph Nunez. Morris water maze experiment. J Vis Exp; 2008, (19): 897.
74. S.k.kulkarni (1999). Hand book of experimental pharmacology. Vallabh parkashan. 1999, ISBN: 81-85731-12-8.
75. Burger c, fischer DR, Cordenunzzi DA, de Borba Batschauer AP, Filho VC, dosantos soares AR. Acute and sub acute toxicity of the hydroalcoholic extract from wedelia paludosa (acmela brasiliensis) (Asteraceae) in mice. J. pharm.pharmaceut.Sci; 2005, 8: 370-373.
76. Sallie R, tredger J.M and Williams R. Drug and liver part 1: teating liver function. Biopharm. Drug dispos.1991, 12:251-259.
77. Henry R J., cannon D C and winkelman J W. Clinical chemistry principles and techniques, 11th ed. Happer and row publishers, new York.p.1629,1974.
78. Pearlman f.c and lee R.T.Y. Detection and measurement of total bilirubin in serum with use of surfactants as solubilizing agents. Clinical chemistry 1974, 20:447-453.

79. Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur.J.Biochem 1974; 47: 469-474.
80. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione stransferase activities in rat lung and liver. Biochimica et Biophysica Acta;1979, 582: 67-78.
81. Kuldeep s sandhu, avtar c rana. Evaluation of anti Parkinson's activityof nigella sativa (kalonji) seeds in chlorpromazine inducedexperimental animal model. NT J Pharm Pharm Sci, Vol 5, 2013, Suppl 3, 884-888.
82. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem.1951, 193: 265-275.
83. Okhawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry; 1979 95: 351-358.
84. Luck H. Methods of enzymatic analysis edited by Hans Ulrich Bergmeyer. 2ndEd. Academic press New York and London:1965, 885-890.
85. Bernt E, Bergmeyer. HU.L-Glutamate UV-Assay with glutamate dehydrogenase and NAD.Methods of enzymatic analysis edited by Hans Ulrich Bergmeyer.2ndEd.Academic press New York and London;1965, 1704-1708.
86. Sivaramanand P.Muralidaran Sedative and anticonvulsant activities of the methanol leaf extract of Ficushispida Linn.D..Drug Invention Today, 2009, Vol.1.Issue 1, pp-23-27.
87. Oswald C, smits SHJ, Ho "ing M,sohn-bo" sser L, Dupont L,Le Rudulier D,et al. Crystal structures of the chloline/acetylcholine supstrate-binding protein Cho X from sinorhizobium meliloti in the liganded and unliganded-closed states.j Biol chem; 2008, 283:32848-32859.
88. Sudhakar Pachiappan, Suchithra K. B, Suganeswari M and Preetu Shukla K.M. Combination therapeutic influence of aspirin and Rivastigmine on scopolamine induced alzheimer's in Wistar rat. Volume 6, Issue 8,2017, 1064-1075.
89. Hong Can Zhu, Jing Zhao, Chang Yue Luo, and Qian Qian Li. Gastrointestinal Dysfunction in a Parkinson's disease Rat Model and the Changes of Dopaminergic, Nitric Oxidergic, and Cholinergic Neurotransmitters in Myenteric Plexus. J Mol Neurosci; 47(1):2012, 15–25.

90. Pierre J Blanchet. Veronika Kivenko Drug-induced parkinsonism: diagnosis and management. Journal of Parkinsonism and Restless Legs Syndrome: 6, 2016.
91. Kawada T, Suzuki S. Depressive state, aging, and prevalence of snacking: a preliminary study. Psychogeriatrics 2011, 11:247–8.
92. Andrea Kistner, Eugénie Lhommée and Paul Krack (2014). REVIEW ARTICLE Mechanisms of body weight fluctuations in Parkinson's disease. Volume 5 |2014\ Article 84.
93. Madhavan Nampoothiri, Jessy John, Nitesh Kumar, Jayesh Mudgal, Gopalan Kutty Nampurath, and Mallikarjuna Rao Chamallamudi. Research Article Modulatory Role of Simvastatin against Aluminium Chloride-Induced Behavioural and Biochemical Changes in Rats. Behavioural Neurology. 210169, 2015, 2915, 9 page.
94. Pietro Mazzoni, Britne Shabbott, and Juan Camilo Corte. Motor Control Abnormalities in Parkinson's disease. Cold Spring Harb Perspect Med; 2012, 2:a009282.
95. Natalia A. Krivova, Olga B. Zaeva and Valery A. Grigorieva. Effect of a water-maze procedure on the redox mechanisms in brain parts of aged rats. Frontiers in Aging Neuroscience |2015\ Volume 7 | Article 29.
96. Sun CC, Luo FF, Wei L, Lei M, Li GF, Liu ZL, LE WD, Xu PY. Association of serum uric acid levels with the progression of Parkinson's disease in Chinese patients. Chin Med J (Engl).125, 2012, (4):583-7.
97. Shivaraj Gowda, Prakash B. Desai, Vinayak V. Hull, Avinash A K. Math, Sonal N. Vernekar, Shruthi S. Kulkarni (2009). A review on laboratory liver function tests.
98. Dhanasekaran. Sivaraman, ratheesh kumar K.S, and palayan muralidaran. Effect of ethanolic seed extract of Mucuna Pruries (L.) DC.VAR.UTILIS on haloperidol induced tardive dyskinesia in rats. Vol. 3, 2010, 0976-044X.
99. Shalavadi M.H, Chandrashekhar V.M, Avinash S.P, Sowmya C, and Ramkishan A. Neuroprotective activity of *Stereospermum suaveolens* DC against 6-OHDA induced Parkinson's disease model. Indian J Pharmacol; 44(6): 2012, 737–743.
100. Rasheed A.S, Venkataraman S, Jayaveera K.N. Evaluation of toxicological and antioxidant potential of Nardostachys jatamansi in

reversing haloperidol-induced catalepsy in rats. International Journal of General Medicine: 2010, 3 127–136.

101. Stocks j, gutteridge jmc et al., assay using brain homogenate for measuring the antioxidant activity of biological fluids. Clin sci., Belgrade 2007; 59 (1): 29-36.

102. Ambani LM, Van Woert MH, Murphy S. Brain peroxidase and catalase in Parkinson disease. Arch Neurol. 1975, ;32(2):114-8

103. Yutaka KASUYA, Tetsuro URUSHIDANI and Susumu OKABE. effects of various drugs and vagotomy on indomethacin-induced gastric ulcers in the rat. Japan j. Pharmacol, 1979 29, 672.

104. Ellman, GL.; Courtney, D K.; Andreas V.; Featherstone, R M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. , 7,1 961, 88–95.

105. Jason R. cannon, victor Tapias, hye mee na, Anthony S. honick, Robert E. drolet, Timothy Greenamyre. J. A highly reproducible rotenone model of parkinson's disease. Neurobiology of disease 34; 2009, 279-290.

106. Humera Khatoon, Rahela Najam, Talat Mirza and Bushra Sikandar Beneficial anti-Parkinson effects of camel milk in Chlorpromazine-induced animal model: Behaviou ral and histopathological study. Pak. J. Pharm.Sci., Vol.29, No.5, September 2016, pp.1525-1529.